UNIVERSITY OF SOUTHAMPTON

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Institute of Sound and Vibration Research

Otoacoustic Emissions and Hearing Threshold

by

Amanda Jane Hall

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ABSTRACT

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OTOACOUSTIC EMISSIONS AND HEARING THRESHOLD

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Cochlear amplification is essential for the highly sensitive auditory system in humans, and is also implicated in otoacoustic emission (OAE) generation. This thesis is based on the premise that over the range of hearing up to and including mild sensorineural hearing loss there is a close relationship between OAE and hearing threshold level (HTL), mediated through the cochlear amplifier (CA). Alternatively, other factors may be important for HTL and OAE generation e.g. inner hair cells and cochlear inhomogeneities, and therefore there is a weak relationship between OAE and HTL. Previous research on human subjects has shown only moderately significant correlations between OAE and HTL. These studies have mostly been cross-sectional with OAE measured over a narrow range of stimulus parameters. Few studies have examined longitudinal changes in OAE and HTL, and even fewer have compared the results of cross-sectional and longitudinal experiments measured using the same equipment and stimulus parameters. Most studies have concentrated on OAE level, and have studied either transient evoked (TE) or distortion product (DP) OAE but not both.

This thesis explores two hypotheses. Firstly that the moderate correlations between OAE and HTL reported in the literature are a result of a poor choice of OAE measures. OAE measures that take into account the level dependency of cochlear amplification are expected to have a higher correlation with HTL. Secondly, that the moderate correlations between OAE and HTL in cross-sectional studies are a result of inter-subject and inter-ear factors unrelated to cochlear amplifier function (e.g. ear canal and middle ear factors). The correlation between OAE and HTL is expected to increase in longitudinal experiments where these factors are constant within-subjects and within-ears across conditions. This thesis addresses these issues by investigating the relationship between OAE and HTL in human subjects with normal hearing or mild sensory hearing loss in both cross-sectional and longitudinal studies. A wide range of OAE measures were investigated. Differences in OAE between subjects with different HTL status were examined by cross-sectional study, while longitudinal changes in OAE were studied within subjects experiencing a temporary hearing loss due to aspirin consumption. Both TE and DPOAE were measured and the data were interpreted within the general framework of OAE generation.

In the cross-sectional study, forty-three subjects with a range of HTL were tested. TE and DPOAE were measured across a range of frequencies and stimulus levels. The longitudinal study involved 17 subjects taking aspirin for three days while OAE were measured over the course of seven days (pre-, peri- and post-aspirin). Correlation and linear regression analyses were performed to investigate the relationship between OAE and HTL in both experiments. Identical measures were obtained in the cross-sectional and longitudinal, which allowed the two studies to be compared directly.

The results of the cross-sectional study relating OAE and HTL were in accordance with previous studies, and showed a low to moderate correlation between the variables. OAE measures explained up to 60% of the variance in HTL. DPOAE measures explained more of the variance than TEOAE. The hypothesis that a wider range of OAE measures would increase the correlation with HTL was not upheld. In the longitudinal study, changes in HTL were small with a maximum threshold shift of 20 dB. Group analysis showed similar relationships between change in OAE and change in HTL to those in the cross-sectional study, with similarly low correlations. The hypothesis that longitudinal changes in OAE would show a higher correlation with HTL than cross-sectional differences was not upheld. However analysis of individual data showed highly significant correlations between changes in OAE and HTL for some subjects and ears, but not all. High correlations were obtained for both TE and DPOAE. Approximately 50% of ears showed more than two significant correlations between OAE and HTL variables, varying across frequency. This indicates a close relationship between the change in OAE and the change in HTL for some ears, although it was not possible to identify factors predicting which ears showed the high correlations.

This research shows that addressing the limitations of previous studies does not, in general, improve the relationship between OAE and HTL. However for some ears, when measured longitudinally, changes in OAE accurately reflect changes in HTL. However, this is not true for all ears, and therefore other factors are also important and further work is required to identify those factors. This research suggests that the lack of high correlation between OAE and HTL in cross-sectional studies is at least partially due to inter-subject and inter-ear factors such as ear canal and middle ear effects. This research has direct application to the use of OAE for monitoring changes in hearing in at-risk populations, such as people exposed to noise.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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LIST OF ABBREVIATIONS

ABR	Auditory brainstem response
ANOVA	Analysis of variance
BM	Basilar membrane
CA	Cochlear amplifier
CF	Characteristic frequency
DPOAE	Distortion product otoacoustic emission
DP-gram	Distortion product-gram
FFT	Fast fourier transform
HTL	Hearing threshold level
IHC	Inner hair cell
I/O	Input-output
MET	Mechanoelectrical transduction
MLS	Maximum length sequence
NIHL	Noise induced hearing loss
NTC	Neural tuning curve
OAE	Otoacoustic emission
OHC	Outer hair cell
PTA	Pure tone audiometry
SD	Standard deviation
SFOAE	Stimulus frequency otoacoustic emission
SNR	Signal to noise ratio
SOAE	Spontaneous otoacoustic emission
SPL	Sound pressure level
STC	Suppression tuning curve
TE	Transient evoked otoacoustic emission
TTS	Temporary threshold shift
TW	Travelling wave

PART 1

INTRODUCTORY WORK

Part 1 describes the background work to this thesis. This section includes the literature review, the framework for the thesis and the methodology used for the experimental work.

1 INTRODUCTION

Introduction to the project

The ear is remarkably sensitive and has a wide dynamic range of hearing, arising in part from the cochlear sensory hair cells. There are two types of hair cell, differing widely in function. The outer hair cells (OHC) are responsible for the fine-tuning of the cochlea and are thought to be the site of the physiologically vulnerable, active, mechanical amplification system of the cochlea, known as the cochlear amplifier (CA) (Davis, 1983; Dallos, 1992). The CA improves sensitivity of the ear at low stimulus levels, and has little effect at high stimulus levels. Damage to the CA reduces vibration of the basilar membrane at low-level sounds, and thus increases hearing threshold level (HTL) (Patuzzi et al, 1989; Robles and Ruggero, 2001). The other type of hair cell, the inner hair cell (IHC) is responsible for converting the vibration of the basilar membrane into action potentials along the auditory nerve. Damage to the IHC causes a reduction or absence of neural information to be sent along the auditory nerve, and thus also increases HTL (Pickles, 1988). Cochlear or sensory hearing loss can thus be thought of as a combination of damage to IHC and OHC (Moore and Glasberg, 1997, 2004), although it is rare to have damage to the IHC without damage to the OHC (Borg et al, 1995).

Auditory information is then transmitted along the auditory nerve, via the brainstem to the auditory cortex (Pickles, 1988). The auditory efferent system is also able to alter the output of the cochlea via the OHC (Guinan, 1996). Damage to these parts of the auditory system (i.e. retrocochlear) also results in hearing loss, although damage to these parts of the system is exceedingly rare without damage to the cochlea (Wright et al, 1987; Schuknecht and Gacek, 1993).

Mild sensorineural hearing loss is common and is likely to involve mainly damage to the OHC, whereas at more severe levels of hearing loss, the IHC and neural processes are also likely be involved (Wright et al, 1987; Schuknecht and Gacek, 1993). Noise induced hearing loss primarily affects the OHC, which are more vulnerable to damage than the IHC (Borg et al, 1995). Short term or moderate noise exposure mainly affects the OHC, whereas longer term or more intense noise exposure can damage the IHC and neurones (Patuzzi, 1993).

Otoacoustic emissions (OAE), an emission of sound from the cochlea, are generally acknowledged to be a consequence of the nonlinear, active amplification processes within the cochlea (Kemp, 1986; Kemp, 2002). Recent models of OAE mechanisms include cochlear amplification as fundamental for generation, although cochlear nonlinearities and inhomogeneities are also important (Talmadge et al, 1998; 2000; Lineton and Lutman, 2003a).

The requirement of cochlear amplification for both OAE generation and acute hearing sensitivity suggest that OAE have potential as an alternative to HTL for investigation of cochlear damage in human subjects, for example monitoring changes in hearing over time. Mathematical modelling

studies suggest a strong link between OAE and HTL, mediated through the CA (Talmadge et al, 1998, 2000) yet the precise relationship between OAE and HTL is still not fully understood.

There have been many experimental studies investigating the link between OAE and HTL, but most have shown only a weak to moderate correlation between the two (e.g. Avan et al, 1991; Avan et al, 1993; Gorga et al, 1993a, b; Marshall and Heller, 1996; Kim et al, 1996). Although different OAE parameters have been studied, most research has concentrated on OAE amplitude obtained using high stimulus levels. There is a need for detailed investigation of other OAE measures obtained across a range of stimulus levels. Recently more complex measures such as OAE input-output functions (I/O) and suppression tuning curves (STC) have been proposed to take into account the level dependency of the CA, and these may have a higher correlation with HTL (Dorn et al, 2001; Pienkowski and Kunov, 2001). There is a need to investigate these fully in human subjects.

Most experimental studies have been cross-sectional and the moderate correlations obtained in these studies may be due to high inter-subject variability, for example differences in external and middle ear characteristics between subjects that affect OAE and HTL differently. There have been few longitudinal studies to determine whether differences in OAE between subjects are similar to changes that occur within subjects, and there is a need to investigate this further.

There have also been few studies comparing transient evoked (TE) and distortion product (DP) OAE in human subjects. TE and DPOAE have different generation mechanisms and changes/differences in HTL are likely to affect the two OAE types in different ways (Shera and Guinan, 1999). There have been few comparisons of TE and DPOAE measured within the same subjects to determine which has the higher correlation with HTL (e.g. Gorga et al, 1993b; Berninger et al, 1995; Marshall et al, 2002). Therefore there is a need to measure TE and DPOAE concurrently within subjects, when investigating differences in HTL between subjects and also in subjects undergoing a temporary hearing loss.

This thesis is based on the premise that OAE and HTL are closely related because OAE reflects OHC function, and mild sensorineural hearing impairment is predominantly due to OHC loss. If this is correct, a high correlation is expected between OAE measures and HTL (up to mild levels of sensorineural hearing impairment). Alternatively, other factors may also be important for both HTL and OAE; these may include IHC loss contributing to HTL, and cochlear inhomogeneities contributing to OAE generation. If this is true, a low to moderate correlation is expected between OAE and HTL.

Two hypotheses will therefore be tested. The first is that the moderate correlations of previous studies are a result of a poor choice of OAE and HTL measures. It is postulated that the underlying relationship between OAE and HTL is close, but is compromised as a result of the OAE measures used. This hypothesis will be tested here by investigation of a wide range of OAE measures based on simple models, using a range of stimulus levels and stimulus rates. Equipment will be used that

enables OAE to be measured at low signal to noise ratios. The relationship of these OAE measures and HTL will be examined. It is expected that OAE measures that account for the level dependency of the CA will have a higher correlation with HTL than other measures reported in the literature.

The second hypothesis is that the moderate correlation between OAE and HTL arises from inter-subject and inter-ear differences that influence OAE and HTL differently, such as middle ear factors. This will be tested by a longitudinal study investigating changes in OAE and HTL within subjects and will allow individual subject factors to be controlled for. It is expected that longitudinal changes in OAE will have a higher correlation with HTL than cross-sectional differences.

Aims

This thesis aims to explore in human subjects the relationship between OAE and HTL by examination of differences in OAE between subjects with differing HTL and changes in OAE within subjects with changing HTL.

This thesis presents an investigation of the relationship between OAE and HTL, with particular reference to mild sensorineural hearing loss and aims to test the hypotheses described above. A wide range of OAE measures are examined, which are based on simple models of OAE generation taking into account the level dependency of cochlear amplification. Low-level stimuli and increased stimuli rate are used, as well as equipment with a low noise floor that enable measurement with low stimulus levels. Cross-sectional differences and longitudinal changes in OAE and HTL are investigated: differences in OAE are examined in subjects with a range of HTL. Changes in OAE are examined in subjects undergoing a temporary hearing loss from aspirin consumption. Both TE and DPOAE are measured concurrently.

Contributions to knowledge

The main contributions are:

1. Investigation of OAE measures and their relationship with HTL

The hypothesis was tested that OAE measures that take into account the level dependency of cochlear amplification have a higher correlation with HTL than other measures. The relationship of OAE measured across a range of stimulus levels with HTL was assessed. In general, OAE measured with lower stimulus levels had a higher correlation with HTL. Other OAE measures based on simple models of OAE generation did not markedly improve the correlation with HTL. DPOAE showed a higher correlation with HTL than TEOAE. Many of the OAE measures investigated, for example those evoked using a high stimulus rate, had higher test-retest repeatability than previous measures

but there was no improvement in the correlation with HTL than those reported in the literature (e.g. Dorn et al, 2001).

Therefore the hypothesis that the use of more complex OAE measures would improve the correlation with HTL was not supported.

2. Longitudinal changes in OAE and HTL in human subjects

This thesis investigated concurrent changes in TEOAE, DPOAE and HTL while cochlear function was disturbed by aspirin ingestion. The hypothesis was tested that longitudinal changes in OAE would have a higher correlation to changes in HTL than cross-sectional differences. Taking the pooled results of all subjects and ears there was a moderate correlation between the changes in OAE and HTL. The strength of the correlation was similar to the cross-sectional study and the hypothesis that longitudinal changes in OAE would increase the correlation with HTL was not supported fully. However for approximately 50% of ears there was a higher correlation between the changes in OAE and HTL for some frequency combinations, which were much higher than for the cross-sectional study results. For these ears the hypothesis holds. It was not possible to predict from other measures the subjects and ears that showed high correlations and therefore further work is required to develop a model suitable for all.

TE and DPOAE changes were also compared. The changes in TE and DPOAE with aspirin were similar overall and within individuals in their correlation with HTL.

By accounting for individual differences in the longitudinal design of this experiment, it was shown that for a proportion of subjects and ears that OAE and HTL are highly correlated, as supported by the simple models underlying this thesis. However for other subjects and ears there are other factors that reduce the correlation between OAE and HTL, indicating that more complex models are required to encompass all subjects.

Three publications have resulted from this thesis to date:

Hall AJ, Lutman ME. Novel methods for early identification of noise-induced hearing loss. Audiology 1999; 38: 277-280.

Lutman ME, Hall AJ. Novel methods for early identification of noise-induced hearing loss. Health and Safety Executive Contract Report 2000; CRR261/2000.

Hall AJ, Lutman ME. The effect of aspirin on human cochlear amplifiers. British Journal of Audiology 2001; 35: 133-135.

Overview of thesis

The thesis is divided into two parts: part 1 contains the background to the thesis and the introductory work, and part 2 describes the experimental work. In Part 1 following this introductory chapter, there is a review of the literature in Chapter 2. This includes a brief review of cochlear anatomy and mechanics and describes the cochlear amplifier and its relationship with HTL. There is a review of the current literature regarding the generation of otoacoustic emissions and the interaction with the CA. The generation of both TE and DPOAE is discussed and a comparison of the generation mechanisms of the two types of OAE is made. Finally a discussion of the relationship between OAE generation and HTL is given and the current gaps in knowledge are identified. Chapter 3 gives the rationale for the thesis. This includes a framework relating the OAE and HTL and sets the context for the experimental work. The aims and objectives are explicitly stated. The general methodology is described in Chapter 4.

Part 2 of this thesis describes the experimental work. Chapter 5 describes the cross-sectional study, examining differences in OAE from a group of subjects with a range of hearing levels. Chapter 6 describes the longitudinal study, relating changes in OAE from a group of subjects undergoing a temporary hearing loss. In Chapter 7, the results of the cross-sectional and longitudinal studies are compared. The results are discussed and conclusions derived in Chapter 8.

2 BACKGROUND

2.1 THE COCHLEA

The human ear is remarkably sensitive and can detect sound across the intensity range 0 to 120 dB and across the frequency range 20 Hz to 20 kHz. These abilities of the ear are only possible on account of the highly nonlinear structure of the sensory organ of hearing, the cochlea, which amplifies weak sounds and extends the lower intensity limit of hearing.

This chapter reviews the mechanics of the cochlea and the cochlear amplifier (CA). The role of the IHC and the efferent system is also described.

2.1.1 Cochlear anatomy

The cochlea is a fluid filled structure, divided by a partition called the basilar membrane (BM). The BM has a non-uniform structure, with varying stiffness and mass along its length. It becomes wider and less stiff from the base to the apex, with a continuous reduction in mass. The cochlear structure is coiled around a central axis called the modiolus.

The sensory cells, the inner and outer hair cells are located on the BM, within a structure called the organ of corti. The inner hair cells (IHC) are flask shaped cells, and form one row towards the modiolus from the tunnel of corti. On the apex of the IHC are stereocilia, small hair-like projections. There are approximately 3500 IHC in a human ear, and they receive approximately 90% of the afferent innervation.

The outer hair cells (OHC) are column shaped cells and form three rows further from the modiolus than the tunnel of corti. The OHC have their stereocilia organised in a V or W shape. The stereocilia are graded in length and connected to adjacent stereocilia by tip links. There are approximately 12,500 OHC in the cochlea, four times the number of IHC. They receive only 10% of afferent innervation and 90% of the efferent innervation.

2.1.2 Cochlear mechanics

A sound stimulus entering the ear creates a pressure wave within the cochlear fluids, which in turn generates a displacement wave along the BM. This latter wave, known as a travelling wave (TW), originates at the base of the membrane and travels along to the apical end. The TW grows in amplitude and decreases in wavelength as it moves apically along the membrane, until it reaches maximum amplitude at a characteristic place along the BM, determined by the frequency of the wave. The variation in the physical properties of the BM along its length enables it to function as a spatial frequency analyser, where each frequency is represented at a characteristic place, determined mainly by the stiffness of the membrane. The highest frequencies of sound cause maximum displacement at

the most basal end nearest the stapes, and as frequency decreases so the characteristic place of maximum displacement moves apically along the BM.

At the characteristic place, the corresponding frequency is known as the characteristic frequency (CF). Beyond the CF, the energy is dissipated, TW amplitude declines rapidly and there is no further apical vibration of the membrane.

Von Békésy (1960) was the first to make measurements of BM vibration in humans. He used cadavers through which he was able to demonstrate the spatial-frequency tuning of the cochlea. His original measurements of BM vibration amplitude indicated a passive, linear increase in TW amplitude with stimulus intensity. These results were not consistent with the fine-tuning and sensitivity that were known to exist from behavioural studies of hearing. They were in fact measurements of a passive cochlea, suggesting that further active physiological processes as well as passive cochlear mechanics contribute to sound detection in live humans.

It was not until 1971 that equivalent measures to those made by von Békésy were obtained from healthy cochlea in vivo (Rhode 1971, 1978; Sellick et al, 1982). These studies demonstrated the finely tuned nature of the cochlea and showed that at low intensity levels, the BM is sharply tuned at the CF. At high intensity stimulus levels, the response of the BM resembled that of von Békésy's passive cochlea. The relationship of BM amplitude with stimulus intensity was also shown to be nonlinear. Johnstone et al (1986) showed a greater increase in BM amplitude at the lower intensity levels compared to the higher levels. The relationship increase was 0.2 dB/dB at the mid-intensity range. Work by Ruggero et al (1997) showed that below a stimulus level of 20 dB SPL, BM growth is linear with a growth of 1 dB/dB, and compressive above this level with a growth rates varying from 0.2 to 0.5 dB/dB. It is possible that BM growth may also be linear at high intensity levels, but it is difficult to measure cochlear function at these levels without damaging the cochlea.

Figure 2-1 shows examples of normalised isointensity curves measured at different intensity levels from normal hearing cochleae at a CF of 10 kHz. As frequency is increased above the CF, the BM at the characteristic place rapidly loses sensitivity. As frequency is decreased, the sensitivity at the characteristic place decreases less rapidly, and reaches a broad tail at the lower frequencies. Comparison of the isointensity curves measured at low and high intensity levels show that fine-tuning is not maintained at the high intensity levels, and the curves at high levels resemble the passive case described above.

Measures of BM vibration against stimulus intensity show nonlinear growth of vibration at the CF. At frequencies below the CF, the plot shows a linear increase in vibration and resembles the passive case described above.

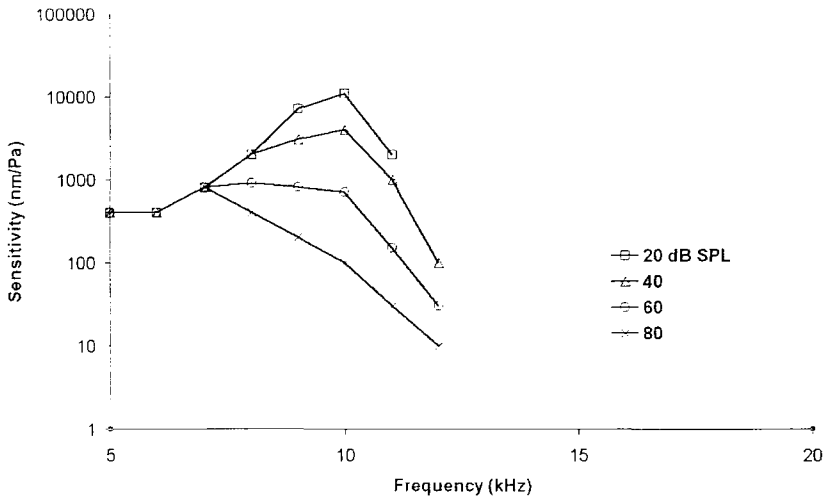


Figure 2-1: Normalised isointensity curves showing the difference in sensitivity of BM responses to tones as a function of frequency and intensity (adapted from Robles and Ruggero, 2001). The curve derived from the lowest stimulus level shows fine-tuning. The CF in this example is 10 kHz.

2.1.3 The outer hair cells and the cochlear amplifier

Fine-tuning of BM vibration is a property of healthy cochleae and is physiologically vulnerable. OHC are generally acknowledged to be responsible for this fine-tuning mechanism; studies have shown that loss of OHC results in significant hearing loss (Ryan and Dallos, 1975) and that the tuning properties of the BM depend upon the health of the OHC (Khanna and Leonard 1986). OHC have little afferent innervation and have motile properties.

The stereocilia on the sensory OHC are coupled together by tip links. They are joined to adjacent stereocilia, as well as to those in front and behind. Movement of the stereocilia group stretches these links. Stimulation of the hair cell bundle in the direction of the tallest stereocilia results in depolarisation of the cell. At threshold this movement is only ±0.3 nm (Hudspeth, 1989). Deflection of the stereocilia is thought to open mechanically gated channels on the hair cell located on or near tips of stereocilia. It is not known exactly how this occurs but they are possibly stretch activated by the tip links that connect the stereocilia (Hudspeth, 1989). These channels are called mechanoelectrical transduction (MET) channels and cause an increase in the influx of potassium ions to the cell down the steep K^+ voltage gradient from the endolymph fluid into the hair cell. This high influx of positive ions depolarises the receptor potential of the cell. When the stereocilia are deflected in the reverse direction, the potassium channels that are open at rest are shut, causing the receptor potential to become hyperpolarised. The further the stereocilia are deflected, the higher the numbers of MET channels are opened. Depolarisation of the receptor potential causes the OHC to contract and shorten, whilst hyperpolarisation causes the cell to elongate (Zenner, 1988). OHC are able to generate opposing forces (Brownell, 1990), and the gene *Prestin*, which codes for a motor protein, has recently

been identified in the OHC (Zheng et al, 2000; Dallos et al, 2002). Prestin is located in the plasma membrane of the OHC, and is thought to be responsible for OHC motility, which can generate forces at frequencies up to approximately 20 kHz.

OHC are thus generally acknowledged to be the site of an active, mechanical, feedback process (Dallos, 1992). These active processes were first termed the "cochlear amplifier" by Davis (1983). The exact mechanism of cochlear amplification is uncertain, and there are currently two theories (Withnell et al, 2002). The first is that the hair cell receptor potential drives a motile process. These motility changes of the OHC force the BM to vibrate more strongly, feeding in energy. As the hair cells are alternately depolarised and hyperpolarised, so the OHC shorten and lengthen on a cycle-by-cycle basis. The contractile properties of the OHC, which cause them to elongate and contract in response to sound, allow them to alter the mechanics of the basilar membrane. The second theory is that there is a motor in the hair cell bundle (Withnell et al, 2002). As the OHC bundle is deflected, the opening of the transduction channels causes the hair bundle to move further in the same direction. When the channels close, they move in the opposite direction. If the gating moves in phase with the sound, then the vibration of the BM is amplified. This may be the mechanism in non-mammals.

Whatever the mechanism of cochlear amplification, the outcome is a large numbers of OHC working as a unit generate force, injecting energy into the travelling wave. These active forces enhance vibration by partially or wholly cancelling resistance; this cancellation can be considered as negative damping (Patuzzi, 1993). The time course of these events is microseconds. The location of the cochlear amplifier (for a particular stimulus frequency) is thought to be at a point just basal of the TW peak (Robles and Ruggero, 2001). At this point along the BM, the activity of the OHC is maximal. At other places along the BM, the CA is minimally active.

The CA force generation mechanism saturates with increasing intensity. It is maximally active to low-level sounds, enabling an increase in BM sensitivity to these stimuli. The relationship between the CA and sound level is highly nonlinear, and as sound level increases, the force generation from the CA decreases, until it eventually saturates at moderate sound levels. Thus the vibration pattern of the TW changes from an active pattern at low stimulus levels to a passive pattern at high levels.

Vibration of the BM in response to sound is therefore composed of the passive vibration of the membrane combined with the active response of the CA. At low intensity levels the CA contributes to a large proportion of the vibration of the TW. At higher levels the active CA is less important, and the TW is largely made up of the passive vibration of the basilar membrane.

The efficiency of the CA is characterised by its gain, which can be related to the energy injected by the OHC. The gain contributions to the TW are maximal at the lower stimulus levels and as stimulus level increases the CA starts to saturate until its contribution is minimal at high stimulus intensities. Thus the effective gain of the CA at the higher levels becomes zero. This is known as *compressive non-linearity* and explains the nonlinear vibration pattern of the BM. CA gain as defined by Ruggero et al (1997) is "the difference (in dB) between the responses to low-level CF tones and the peak responses to high-level tones".

2.1.3.1 The effect of damage to the outer hair cells

As described previously, damage to OHC results in a reduction in sensitivity, increases the linearity of BM vibration and results in a broadening of BM tuning curves (Ryan and Dallos, 1975; Khanna and Leonard 1986).The tuning curve from the BM of a damaged cochlea loses its finely tuned tip and shows much broader tuning than a normal curve. It is similar to the tuning curve derived from a normal hearing cochlea using moderate or high-level stimuli. The tuning curve correspondingly shows an increase in threshold at the characteristic place.

Ruggero and Rich (1991) examined the changes in the mechanical response of the basilar membrane on injection of furosemide in the chinchilla. They showed a reduction in basilar membrane vibration, which was greatest at the low intensity stimulus levels at the CF. There was little effect at high stimulus levels or at frequencies away from the CF. This is consistent with a reduction in the gain of the CA. Damage to the OHC therefore results in a reduction in compressive nonlinearity and a broadening of the BM tuning curves.

Several studies have investigated the relationship between the OHC and hearing sensitivity. Ryan and Dallos (1975) showed that absence of cochlear OHC resulted in behavioural auditory thresholds. The hearing loss measured was approximately 40 to 50 dB. This provided evidence that a change in the mechanical sensitivity of the cochlea affects hearing threshold and is consistent with the accepted value of the maximum gain of 60 dB added by the cochlear amplifier in humans (Robles and Ruggero, 2001).

There is evidence that changes in the active assistance from the OHC relate directly to changes in HTL. Patuzzi et al (1989a) exposed guinea pigs to loud noise, and correlated the change in neural sensitivity (as measured by the compound action potential (CAP)) and the change in amplitude of the cochlear microphonic, which measures the integrity of the OHC. They found a high correlation between the two, with an increase in the CAP highly correlated with a reduction in the amplitude of the cochlear microphonic following exposure to noise. The authors concluded that the disruption to OHC function was probably due to inactivation of the MET channels of the OHC. This disruption of OHC led to a change in the vibration of the basilar membrane. The relationship between the change in threshold and the microphonic over the normal to mild hearing loss region was approximately 1:1.

Patuzzi et al (1989b) further investigated the effect of other agents on the relationship between the CAP and the cochlear microphonic. They investigated phenomena that directly affected the OHC such as two-tone suppression, and found a similar relationship between a change in the cochlear microphonic and the CAP as in their previous study (Patuzzi et al, 1989a) suggesting a direct relationship between the OHC transduction process and hearing threshold. Other treatments such as cooling, which is likely to affect neural transmission but have little effect on mechanical vibration showed no change in the cochlear microphonic but a large change in CAP threshold as expected.

Liberman et al (2002) tested the hypothesis that the electromotility of the OHC is the basis of the cochlear amplifier and that reducing the electromotility directly affected hearing threshold. Liberman et al targeted the *Prestin* gene in mice and created animals heterozygous for the gene and also with the prestin gene deleted, known as 'prestin null'. They compared electromotility of OHC, ABR threshold and DPOAE threshold of these mutant mice with wildtype mice (prestin homozygous). OHC electromotility was measured in-vitro, and prestin null mice had no measurable motility. Prestin heterozygous mice showed half the length changes in OHC of the homozygous wildtypes. The ABR thresholds of prestin null mice were 45-60 dB greater than the wildtypes, whereas the thresholds of the heterozygous mice were 1-8 dB greater. DPOAE thresholds in the prestin mice were 45-55 dB greater than the wildtypes, and that of the heterozygous mice were 3-6 dB greater. They concluded that in-vitro electromotility of OHC and in-vivo hearing sensitivity is directly related, and proposed that the electrically induced changes add linearly to BM vibration. In mice heterozygous for the gene, which showed half the normal length change in OHC, there was the predicted 6 dB drop in sensitivity.

These studies provide evidence that a change in the active force delivered by the OHC to enhance the vibration of the BM is accompanied by a change in hearing threshold.

2.1.4 Inner hair cells

Inner hair cells are the sensory cells of the cochlea and thus have the majority of afferent innervation (Dallos, 1996). The stereocilia of the IHC are coupled by tip links, and in a similar way to the OHC, deflection of the hair cell bundle by motion of the cochlear fluid causes a change in the receptor potential of the cell. This change in receptor potential sets off a process that releases neurotransmitter onto afferent synapses.

The IHC convert the mechanical sound energy into electrical energy as neural impulses, and therefore are responsible for converting the vibration of the basilar membrane into action potentials along the auditory nerve (Pickles, 1988). Figure 2-2 summarises the cochlear transduction processes of the IHC and OHC.

IHC have similar tuning curves to OHC. A damaged IHC has a tuning curve that retains its shape, but for which threshold is raised (Pickles, 1988). Damage to IHC can result in smaller neural response than usual to a particular BM vibration and thus increases HTL.

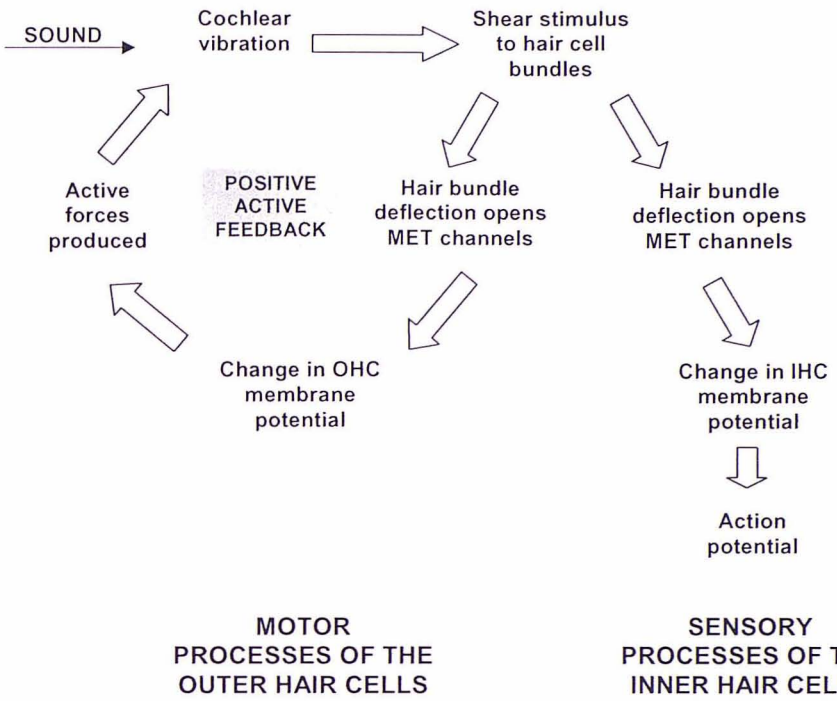


Figure 2-2: Diagram illustrating the transduction processes within the cochlea. This shows the motor processes of the OHC, which feed force into the travelling wave, and the sensory processes of the IHC, which generate an action potential. Key to abbreviations – MET: mechanoelectrical transduction, IHC: inner hair cell, OHC: outer hair cell. Adapted from Patuzzi (1996).

2.1.5 Auditory neural processes

The afferent innervation from the IHC is transmitted along the auditory nerve, via the auditory brainstem to the auditory cortex (Pickles, 1988). Processing of the signal is continued all along the pathway up to the auditory cortex.

2.1.6 The efferent system

As well as the afferent system, which transmits sound from the cochlea to the brain, the brain is also able to influence the output of the cochlea via the efferent system. The olivocochlear efferent system originates in the brainstem and innervates the Organ of Corti. It can be classified into the lateral and medial efferent system, which have two different functions. The lateral efferent system innervates the IHC, and the medial efferent system innervates the OHC. The lateral system is unmyelinated and

terminates on the dendrites of the IHC, whereas the medial system is myelinated and terminates directly on the OHC (Dallos, 1992; Guinan, 1996).

Stimulation of the medial efferent system appears to affect motility of the OHC, and results in a reduction of the cochlear compound action potential and OAE level (Hill et al, 1997; Veuillet et al, 1999). The effects of this stimulation are greatest at lower stimulus levels. It is therefore generally considered that one of the functions of the medial efferent system is to reduce the gain of the cochlear amplifier (Guinan, 1996). The efferent system may have several roles with humans: It may be to shift the dynamic range of the auditory system allowing adaptation for different listening situations. It may also be to improve detection of stimuli in noise. Thirdly it may be used to protect the ear from damage to high intensity sounds. (Guinan, 1996).

Within this thesis, the effect of the efferent system on the auditory system is included within the overall concept of the cochlear amplifier. No attempt is made to assess the role of the efferent system in experimental subjects.

2.1.7 Hearing threshold and hearing loss

Hearing level depends on the IHC and OHC, central auditory processes and the efferent system. A change in hearing threshold can be due to any change in the hearing pathway including the OHC, IHC, neural pathways or any combination of these.

This thesis focuses on mild sensorineural hearing loss and the assumption is made that this level of hearing impairment is primarily due to OHC dysfunction. In Moore and Glasberg's model of loudness perception (1997; 2004) they describe cochlear hearing loss as a combination of IHC hearing loss and OHC hearing loss. The OHC are more susceptible than the IHC (Dallos, 1992; Borg et al, 1995) and it is rare to find damage to the IHC without damage to the OHC (Wright et al, 1987; Schuknecht and Gacek, 1993; Borg et al, 1995). There is evidence of a link between OHC function and HTL (Patuzzi, 1989), particularly for mild levels of hearing loss. Therefore mild sensorineural hearing loss is likely to be primarily associated with OHC loss. However damage to other structures such as the IHC and higher auditory processes cannot be ruled out.

This thesis focuses on mild sensorineural hearing loss, as it is likely that the primary component of the loss is OHC dysfunction. However it is acknowledged that IHC and other components of the auditory pathway may also contribute to the hearing loss.

2.2 OTOACOUSTIC EMISSION GENERATION

This section describes otoacoustic emission (OAE) generation. Many OAE models are based on mathematical models of cochlear mechanics, so a brief description of cochlear mechanics is given. Transient evoked (TE) and distortion product (DP) OAE generation is then described and factors necessary for their generation.

2.2.1 Models of cochlear mechanics: a background to OAE models

Models of OAE generation are based on existing models of cochlear mechanics, which have been in development since Zwislocki in 1948 (de Boer, 1996). These cochlear models have attempted to explain the fine-tuning of the basilar membrane, which arises from the activity of the outer hair cells (OHC) and leads to selective amplification of the travelling wave. Secondly, they aim to explain the nonlinearities of the cochlea, which arise from the nonlinear transduction processes of the OHC.

For modelling purposes, according to de Boer (1996), the cochlea can be simplified as two fluid filled channels, separated into two sections by the cochlear partition (comprising the basilar membrane and the organ of corti). The upper channel consists of the scala vestibuli and the scala media, and the lower channel the scala tympani. The cochlear wave generates antisymmetrical pressure of the fluid in the two channels. In a "classical" cochlea model, longitudinal coupling of elements along the Organ of Corti is ignored (de Boer, 1996), meaning that the mechanics of the cochlear partition can be described by impedance. The impedance of the BM is modelled using terms for stiffness, mass and resistance. This enables the model to generate the response of the BM for different frequencies and locations along the cochlea.

Models of the cochlea can be described as one, two or three dimensional, which refers to the treatment of the fluid movement in the model. A one-dimensional model, also known as the longwave model describes fluid movement in the x coordinate only i.e. along the basilar membrane. A two-dimensional model describes fluid movement in the x and z coordinates only. A three-dimensional model includes movement of the fluid in all directions. The longwave model is commonly used, and otoacoustic emission models are often based on this (de Boer, 1996).

Initial classical models developed by Zwislocki in 1948, and Dallos in 1973 were able to explain the passive response of the cochlea but did not provide the sharply peaked BM vibration responses. Following the experimental work showing the importance of the OHC in BM fine-tuning (Rhode, 1971; Rhode 1978; Khanna and Leonard, 1982), the existence of a cochlear amplifier residing in a region basal to the activity pattern peak became generally accepted (Davis, 1983). Neely and Kim (1986) were the first to introduce a locally active mechanism to the cochlear models to explain the fine-tuning observed experimentally. The model they developed was based on a classical, long-wave,

linear model, with an active mechanism generated by the OHC, to give a force acting directly on the BM. Their model generated results comparable to physiological responses. Altering the gain of the feedback loop in their model reduced the sharpness of the mechanical tuning curves in accordance with experimental data.

Since Neely and Kim published their model, other models have included an active component (see de Boer, 1996 for a review). The active process is modelled as either negative damping or active force generation. Negative damping provides a "cycle by cycle force to the BM in opposite phase with the resistive force produced by the passive damping" (Dallos, 1992). The active force generation method feeds back a reactive force into the BM. The exactitudes of the active generation are unimportant for functioning of the models; rather it is the gain provided that is important for the fine-tuning of the models (de Boer, 1996). According to Kemp (2002), the complexity of the amplification processes means that it has still not been adequately modelled. Current models in use tend to be based on the classical models i.e. long-wave, one-dimensional and include a locally active element with no longitudinal coupling. Nonlinearity is sometimes included.

Models of OAE generation are based on these cochlear models. However the classical cochlear model does not generate OAE, and neither does the classical model with only active processes included (de Boer, 1996). For generation of OAE, cochlear models require the presence of active processes as well as other components, as summarised in Table 2-1. Models include active processes, but additionally for generation of OAE they require reflection sites in the form of inhomogeneities (Zweig and Shera, 1995). Nonlinearities are required for DPOAE and may also be used for TEOAE production (Talmadge et al, 1998; 2000).

Table 2-1: Summary of components required to generate OAE in mathematical models

TEOAE generation	DPOAE generation
Active processes	Active processes
Inhomogeneities	Inhomogeneities
Nonlinearities*	Nonlinearities

* Not essential

2.2.2 TEOAE generation

TEOAE are generated by stimulation of the cochlea with a transient stimulus, in the form of either a tone or a click. Kemp (1978) proposed that TEOAE were generated within the cochlea by reflection of the evoking stimulus from irregularities or perturbations along the basilar membrane, giving an echo that is emitted into the ear canal.

For click evoked OAE, the spectrum of the TEOAE is periodic, with a spectrum period of approximately 0.5 Bark. Models of TEOAE generation have attempted to explain this periodicity: the spatial corrugation model and the model of coherent reflection filtering, both of which are based on the phenomenon of Bragg scattering.

2.2.2.1 Bragg scattering

Bragg scattering is a phenomenon that occurs in any uniform medium, and is best described using the example of crystals. Crystalline structure consists of small, identical perturbations positioned at regular intervals, the spacing of which differs with the type of crystal. If white light (containing all wavelengths) is shone through a crystal, then a monochromatic light at a particular frequency is passed through. The frequency of this light varies with the spacing of the perturbations.

The monochromatic light is produced from scattering of the original white light. The different wavelengths present in white light encounter the crystalline perturbations and this encounter causes them to scatter and generate wavelets. The wavelets all have different phase characteristics and tend to cancel out. However at the one frequency of light that has a wavelength equivalent to the distance between adjacent perturbations, the wavelengths are in phase and summate. This is known as the Bragg condition (where the distance between two perturbations is equal to one wavelength). "As the phase of a wave changes by 1 cycle over the course of the wavelength, then all scattered wavelengths have the same phase and superimpose coherently" (Zweig and Shera, 1995). If the wavelets do not satisfy the Bragg condition, they cancel each other out, and hence no light is passed through.

2.2.2.2 Spatial corrugation models

Strube (1989) hypothesised that the cochlea is spatially corrugated, sometimes described as "the cochlear washboard". He proposed a model in which TEOAE periodicity was explained by the interaction of the evoking stimulus with the regular arrangement of hair cells along the basilar membrane. Through Bragg scattering of the stimulus from a regular pattern of perturbations within the cochlea, TEOAE would be generated with a regular phase pattern.

Spatial corrugation models explain the periodicity in OAE as shifts in phase arising from wave propagation and reflection. As sound is transmitted into the cochlea and reflected at some points on the BM as an OAE, there is a roundtrip shift in the phase of the wave due to propagation into the cochlea, reflection by cochlear perturbations and emission back out of the ear. Strube proposed that

the interaction of the reflectance component with the evoking stimulus is responsible for the periodicity in the spectrum. The regular periodicity of the TEOAE spectrum was interpreted such that the perturbations or scattering sites along the cochlea are arranged in a regular pattern, and this regular pattern accounts for the regular phase shifts.

However, anatomical studies of the cochlea are not consistent with this theory. The cochlea has an irregular pattern, and the distance between perturbations is probably variable: there is no evidence for spatial corrugation.

2.2.2.3 Coherent reflection filtering

Following on from the models of spatial corrugation, Shera and Zweig (1993) and Zweig and Shera (1995) developed a theory called coherent reflection filtering. This was also based on Bragg scattering, but was able to explain regular phase variation in OAE, taking into account the irregular arrangement of hair cells in the cochlea.

Zweig and Shera proposed that a form of Bragg scattering occurs within the cochlea. This cochlear analogue of Bragg scattering occurs a result of the non-uniform medium of the cochlea and the irregularity of the perturbations. The perturbations responsible for Bragg scattering are probably the OHC positioned along the length of the BM. Unlike the perturbations within a crystal, these hair cells are arranged in a dense and irregular fashion.

The forward travelling wave along the BM is reflected from these irregular perturbations, and wavelets are generated from the OHC inhomogeneities along the entire length of the membrane. The wavelets interact but due to their different phase characteristics, combine out of phase and cancel each other out.

However the situation is different at the place of the characteristic frequency of the TW. At this point, the TW undergoes cochlear amplification resulting in a finely tuned, tall and broad wave, in a manner similar to a spatial filter. The area under the TW contains a number of densely positioned scattering sites, and cochlear amplification at this place ensures that wavelets originating here have a larger level than those originating elsewhere. Due to random perturbations, some of the wavelets at the frequency of the TW may satisfy the cochlear analogue of the Bragg condition and summate coherently.

The TW can thus be thought of as a passband filter, capturing coherent wavelets at its characteristic frequency and suppressing those wavelets at all other frequencies, see Figure 2-3. Whereas at all other points along the cochlea the reflected wavelets cancel each other out due to similar amplitude and random differences in phase, at the peak of the TW the wavelets are larger and have the potential to line up in phase and summate coherently or alternatively to cancel one another (Zweig and Shera, 1995).

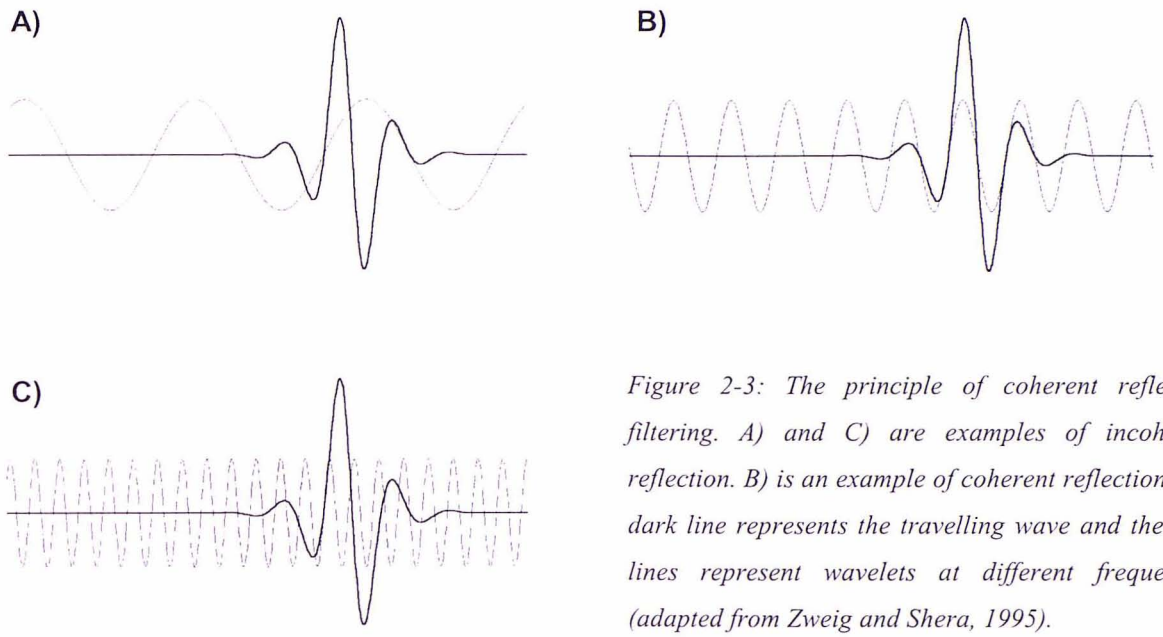


Figure 2-3: The principle of coherent reflection filtering. A) and C) are examples of incoherent reflection. B) is an example of coherent reflection. The dark line represents the travelling wave and the grey lines represent wavelets at different frequencies (adapted from Zweig and Shera, 1995).

For reflection of a forward TW to occur, there is a requirement that the spatial filter is both tall and broad, with a densely packed, irregular arrangement of OHC within the peak. Reflection depends on the average roughness in the area of the maximum activity, and also on the bandwidth of the spatial filter, which is itself related to the width of the activity pattern of the peak region (Talmadge et al, 1999).

The coherence of reflected wavelets in a region of the BM generates a backward TW. This TW is dominated by the frequency of the original TW. The theory of coherent reflection filtering (Zweig and Shera, 1995) has been successfully modelled mathematically: Kalluri and Shera (2001) state that "given 'almost any' arrangement of micromechanical impedance perturbations (i.e. an arrangement with the appropriate spatial-frequency content, such as perturbations that are randomly and densely distributed), a model will produce realistic reflection emissions whenever the peak region of the travelling wave has a slow varying wavelength and an envelope that is simultaneously both tall and broad". The requirement of random and dense perturbations along the BM for TEOAE production is consistent with the fact that TEOAE from small mammals (e.g. guinea pigs), which have a fairly regular arrangement of OHC, are smaller and more difficult to record than in humans. Lineton and Lutman (2003b, c) modelled both the Strube, and Zweig and Shera models, and provided experimental evidence to support the Zweig and Shera model but not the Strube model.

The theory of coherent reflection filtering can explain the phase oscillations of TEOAE with frequency. Unlike the spatial model described previously, which accounts for periodicity as a change in the phase as a single wave is reflected back from regularly spaced points, the coherent filtering

model considers that the backward TW is composed of multiple wavelets originating throughout the cochlea with the coherence dependent on wave shape rather than spacing between perturbations. It is the interaction of these wavelets, which depending on phase either summate or cancel out, and give an emitted TW whose phase changes with frequency. For both models, phase of the reflection TW changes rapidly with frequency, as the place of maximal reflection perturbation changes (Kalluri and Shera, 2001). These types of emissions are also known as reflection-source OAE or place-fixed OAE for these reasons.

Unlike the spatial corrugation models, the theory of coherent reflection filtering provides an explanation for TEOAE generation and the observed periodicity that is consistent with anatomical evidence. For both models, the irregularities and perturbations responsible for reflection are fixed along the basilar membrane, and are not caused by the wave itself. Changing the frequency of the TW moves the wave along the BM, but the perturbations do not move with the wave. Reflection-source OAE are thought to give frequency specific information mainly relating to the site of reflection. The identifying characteristic of a place-fixed emission is that phase is rapidly changing with frequency.

2.2.2.4 Intermodulation distortion

Recently, the role of intermodulation distortion (in addition to linear reflection) in TEOAE production at high stimulus levels has been suggested. Yates and Withnell (1999) measured TEOAE in guinea pigs using high-pass filtered clicks. As well as recording TEOAE at the same frequency as the click, they also recorded components with significant level at lower frequencies than present in the original stimulus. They concluded that this was due to nonlinear interaction of the high frequency stimulus components generating distortion[1]. This distortion generates both a basal TW and an apical TW, which is then reflected as basal TW. Withnell et al (2000) measured the change in TEOAE after noise exposure in guinea pigs. A high-frequency hearing loss was induced using a 12 kHz pure tone at 110 dB SPL and TEOAE were evoked by filtered click stimuli of different bandwidths: 1-5, 1-10, 1-15, 1-18 and 10-18 kHz. These different bandwidths were used to test whether stimulation from the click at the frequency of the hearing loss was important i.e. was the intermodulation distortion arising from the click itself? They showed that when a wideband click stimulus was used (that stimulated the frequency of the hearing loss), sound exposure caused changes in the lower frequency components of the TEOAE (as Yates and Withnell, 1999). However when a filtered click stimulus was used that did not stimulate the area of the cochlea with the hearing loss, the changes in low frequency TEOAE were

[1] Intermodulation distortion products can occur at frequencies above or below the primaries. Knight and Kemp (2001) refer to the lower frequency components (e.g. $2f_1-f_2$, $3f_1-2f_2$) as low sideband components. Yates and Withnell (1999) imply low sideband components.

negligible. They suggest that this provides further evidence for intermodulation distortion in TEOAE generation.

Similarly, Talmadge et al (2000) proposed that nonlinear reflection (arising from distortion components) has a role in predominantly reflection-source OAE (stimulus frequency (SF) and TEOAE) at high stimulus levels. Unlike coherent linear reflection, the original basal nonlinear TW is a wave-fixed phenomenon, where the generation site depends on the position of the peak of the TW. If intermodulation distortion plays a role in TEOAE generation, there will be a contribution to the OAE from frequencies lower than that of stimulation, as well as at the original frequency. The original source of the lower frequencies is in the region where the intermodulating frequencies interact, although there may also be a second source at the place corresponding to the intermodulation product frequency due to reflection of the apical TW. Intermodulation is more likely to be significant at higher stimulus levels, although at the exact level at which distortion influences TEOAE generation is not known.

It can be argued that intermodulation must occur during TEOAE production whenever wavelets of the stimulus at different frequencies are present at the same time at a place on the BM, in exactly the same way as DPOAE (see below). However the magnitude of these intermodulations may be small under most conditions, compared to the OAE components from a simple reflection of a wavelet at a single frequency (see below).

2.2.2.5 TEOAE production

TEOAE generation has been described as superposition of many SFOAE (Kalluri and Shera, 2001). If this is the case, individual frequency components of TEOAE are assumed to arise from direct stimulation of the cochlea with stimuli at the same frequency. This theory assumes that TEOAE are generated solely from linear reflection sources. However, the support for this is not conclusive, and there is evidence (see above) that TEOAE are not only generated from localised 'emission channels' but also have contributions from distributed sources along the cochlea (e.g., intermodulation distortion is also thought to contribute to TEOAE production).

Studies of TEOAE suppression using pure tones generally support the localised model of TEOAE generation (Kemp and Chum, 1980; Tavartkiladze et al, 1994). These showed that introduction of a pure tone during emission generation suppressed the corresponding site of the TEOAE, resulting in a notch at that frequency in the TEOAE spectrum. However, experiments by Sutton (1985) and Withnell and Yates (1998) gave different results, with the suppressor tone also suppressing other frequencies, suggesting a lack of frequency specificity. These latter studies provide evidence that TEOAE are also generated by distributed sources.

Frequency specificity of TEOAE has also been questioned by Avan et al (1997), who showed that subjects with purely high frequency hearing losses had reduced level TEOAE at the TEOAE

frequencies where hearing was normal. They concluded that variation in high frequency hearing contributes to the variation in TEOAE at lower frequencies. This finding may be explained by the contribution of intermodulation distortion to TEOAE generation: in a normally hearing cochlea, distortion is generated at the high frequency end of the cochlea and this travels towards the apex where it is reflected. The high frequency distortion contributes to the overall TEOAE level. A cochlea with a high frequency hearing loss will generate less distortion from the areas with a hearing loss, resulting in a reduction of the overall level of the TEOAE, even if hearing at the lower frequencies is normal. Avan et al (1997) only used a moderately high stimulus level, and did not examine the effect of high frequency hearing loss on TEOAE evoked at lower intensity stimulus levels. At the lower stimulus levels, the involvement of distortion in the TEOAE generation may be lower and the effect of high frequency hearing on TEOAE level may be less important.

2.2.2.6 Cochlear amplification and TEOAE

Coherent reflection filtering, one of the likely mechanisms behind TEOAE generation, requires a 'tall and broad' TW. The tall and broad shape of the TW arises from cochlear amplification at the CF. The width of the CA site is also thought to be important, and may modify the broadness of the TW. A reduction in CA function at the CF is hypothesised to reduce the 'tallness' and increase the 'broadness' of the TW and hence reduce the coherent reflection recorded as a TEOAE, giving a reduction in TEOAE level. Reflection is fundamentally a linear process, and so the relationship between a reduction in TW level and reflection is expected to be 1:1. It is likely that at a particular CA gain level, the TW shape will have changed such that the conditions for coherent reflection are no longer fulfilled. At this gain level, coherent reflection will be negligible and TEOAE will not be recorded. The exact relationship between 'tallness and broadness' of the TW and coherent reflection is as yet unknown.

The effect of a reduction in CA function on the distortion components of the TEOAE is also unknown. At high stimulus levels distortion may dominate TEOAE generation, with contributions from reflection at these high levels being minimal. TEOAE evoked by higher stimulus levels are less sensitive to dysfunction than lower levels (Marshall and Heller, 1996), and this may indicate that distortion is less sensitive to a reduction in CA gain than reflection. Changes in CA function may have a greater effect on the coherent reflection filtering mechanism. In fact Shera et al (2000) used this argument to suggest that reflection-source emissions are likely to be best for probing cochlear function. Kemp (2002) also argues that "CA gain is more strongly represented in place-fixed frequency emissions".

The precise relationship between the CA and OAE generation is not fully understood; neither is the effect of a reduction in CA gain on OAE generation. However the CA is clearly implicated in OAE generation and is at least a modifying factor. CA involvement is potentially different in TE and

DPOAE and this is apparent from the fact that TEOAE are not recorded when hearing threshold level (HTL) is greater than 30 dB, whereas DPOAE can be recorded with HTL up to 60 dB (Probst et al, 1991).

2.2.2.7 Summary of TEOAE generation

There is evidence that TEOAE production is a combination of linear reflection from distributed roughness along the BM, and possible distortion (and subsequent reflection) from cochlear nonlinearities. These relative combinations may depend on the stimulus level. The reflection mechanism is intrinsically a linear process at low stimulus levels. However, increasing stimulus level reduces cochlear amplification and the height of the TW and broadens the activity region, which has the effect of reducing the relative reflectance. Increased stimulus level also shifts the position of the TW basalward, and so changes the frequency position of the reflectance (Talmadge et al, 2000). It is unlikely that there is any further contribution of reflectance at high stimulus levels (Zweig and Shera, 1995). Although the reflection process itself is linear, the nonlinear relationship of the TW with stimulus level explains the nonlinear relationship of the TEOAE level with stimulus level. Intermodulation distortion may also be implicated, but the relative importance of this component may be low for most stimulus conditions.

2.2.3 DPOAE generation mechanisms

DPOAE are typically generated by stimulating the cochlea with two related primary tones, at frequencies f_1 and f_2, with $f_1 < f_2$, and observed as intermodulation distortion. The resultant distortion product can be recorded in the ear canal: the largest is the cubic product $2f_1-f_2$, although others such as $2f_2-f_1$ and f_2-f_1 are also produced. Production of the distortion product $2f_1-f_2$ is mainly discussed here, although generation of $2f_2-f_1$ is also briefly mentioned.

Unlike TEOAE, which are generated predominantly by a reflection source, there is strong evidence that DPOAE have two components that both contribute materially to the emitted response (Brown et al, 1996; Talmadge et al, 1999; Shera and Guinan, 1999). These components are thought to arise from two different sites along the BM; they then mix within the ear canal to give the resultant DPOAE. The mechanisms are (wave-fixed) intermodulation distortion and (place-fixed) coherent reflection filtering, see Figure 2-4.

2.2.3.1 Intermodulation distortion

Intermodulation distortion arises at the place of overlap of the two primary stimuli along the BM. The region of the peak of the f_2 TW envelope is the most important site of overlap. Cochlear nonlinearities associated with the active cochlea give rise to this distortion, which generates both backward and forward TW at distortion product frequencies, the main one being $2f_1-f_2$. Examples of the processes that generate these nonlinearities include the Boltzmann function relating stereocilia displacement and

OHC receptor current, and also the relationship between OHC length change and voltage. The generation of DPOAE depends on the form and degree of these nonlinearities, and these in turn are related to CA function. Interaction of the primaries initiates the new TW at frequency f_{dp}. The TW travelling towards the base is mainly emitted via the middle ear and ear canal to form part of the measured DPOAE, although some energy may be reflected at the stapes.

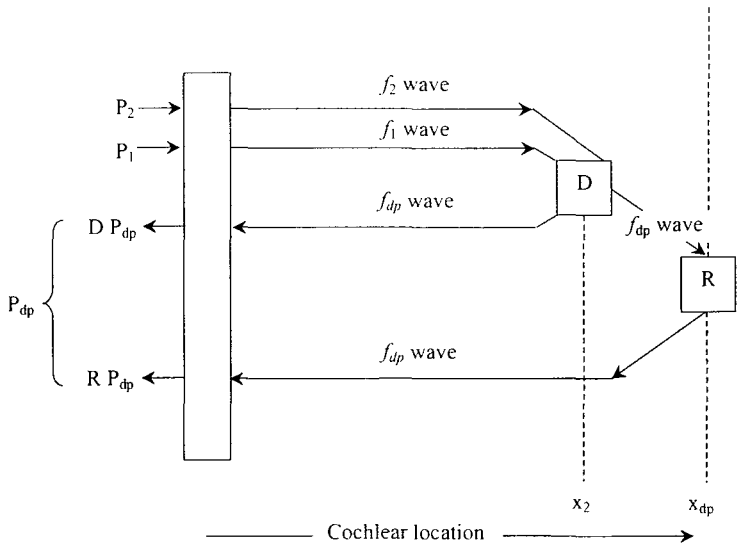


Figure 2-4: Generation of DPOAE (adapted from Kalluri and Shera, 2001). The primary tones f_1 and f_2 interact to generate the f_{dp} distortion wave, which is both transmitted in a basal direction out of the ear and in an apical direction. The apical wave travels to the corresponding place on the BM, where it is reflected to produce a second basal wave. The distortion and reflection components combine to form the distortion product P_{dp}. Key- P_1 and P_2: primary tones, D: distortion, R: reflection.

This distortion source OAE has been described as wave-fixed OAE (Knight and Kemp, 2000) because the emission site is an integral part of the TW. Changing the frequencies of the primary stimuli, while maintaining the ratio f_2/f_1 shifts the TW of the primary stimuli along the BM with a corresponding shift in the nonlinear interaction of the two waves. Due to a condition that is approximated by TW mechanics, known as scaling symmetry, there is little or no shift in the phase of the distortion OAE with changing frequency of the primary waves. This is because the same relative excitation pattern is produced on the BM whatever the frequency, arising from the approximately logarithmic frequency map of the BM. Hence a low or high frequency TW contains the same number of wavelengths.

Distortion OAE have also been labelled as low-latency DPOAE (Knight and Kemp, 2001). This arises because they are generated from positions in the TW basal to the peak, where the TW has not slowed down fully (as would occur at the TW peak). Hence the latency at the instant of the DPOAE generation is relatively low. This contrasts with reflection OAE that are generated at the corresponding TW peak, and have longer latencies.

2.2.3.2 Coherent reflection filtering mechanisms

Part of the distortion product TW travels in an apical direction and reaches the characteristic place of the distortion product f_{dp}. At this point, the TW undergoes coherent reflection filtering, as described earlier for TEOAE. The reflected OAE at this site undergoes cochlear amplification and is transmitted basalward where it then interacts with the distortion component.

These reflection OAE have been labelled place-fixed OAE, as the reflection sites are fixed on the BM, and do not move with the TW (for constant f_{dp}). These reflection contributions have longer latencies than distortion OAE (as they are generated at the TW peak), and are also known as high-latency DPOAE (Knight and Kemp, 2001).

Coherent reflection filtering cannot occur for high sideband DPOAE (e.g. $2f_2–f_1$) in the same way. This is because the distortion product wave cannot propagate in the region of both primaries, as its frequency is too high. Therefore high sideband DPOAE must be generated in the region of the DP frequency characteristic place (in the region of the f_2 envelope) where they are likely to be generated by reflection sites and travel immediately basally (Knight and Kemp, 2000). Alternatively they may be generated at a site apart from the reflectors, and travel apically until they reach the reflection site at which point they travel basally.

2.2.3.3 2f1–f2 generation

The two source components of DPOAE, from different sites along the BM combine to form the DPOAE, the largest of which is at $2f_1–f_2$. It is not yet established what the relative contributions from the two different sites are to the overall DPOAE. Also the contribution of the two sites means that assigning frequency specificity to the resultant distortion product is difficult. Absence of nonlinearity at the overlap site must abolish both the distortion and reflection components. However, absence of 'tall and broad' spatial filtering at the f_{dp} site will result in absence of the reflection component only.

Unmixing techniques have been described in the literature to separate out the two components of DPOAE. These techniques included suppression, the use of pulsed tones and the inverse fast Fourier transform method.

Unmixing using suppression involves first obtaining the unsuppressed DPOAE, then using a third tone at a frequency slightly above f_{dp} to suppress the reflection component, hence recording the DPOAE generated by distortion only. Subtraction of the distortion DPOAE from the unsuppressed DPOAE leaves only the reflection DPOAE (Heitman et al, 1998; Talmadge et al, 1999; Kalluri and Shera, 2001).

Pulsed tones have also been used to unmix DPOAE (Whitehead et al, 1996; Talmadge et al, 1999). One primary tone is held constant while the other is pulsed. This results in DPOAE production that is

also pulsed, and causes the two latency components to separate. As the primary is turned on, the short latency component dominates, and as it is turned off the longer latency component dominates.

Separation of the two components of DPOAE can also be performed using an inverse fast Fourier transform (IFFT). This transforms the data into the time domain and separates out components of the DPOAE with different latencies (Stover et al, 1996; Kalluri and Shera, 2001; Konrad-Martin et al, 2001, 2002; Knight and Kemp, 2000, 2001). An early peak in the IFFT is assumed to represent the low-latency (distortion) component and a later peak to represent the high-latency (reflection) component. The results obtained using IFFT are similar to those obtained using third tone suppression. Additionally, the use of a suppressor tone has been shown to remove the late peak of the IFFT (Konrad-Martin et al, 2001).

Shera and Guinan (1999) state that distortion rather than reflection is the dominant source for DPOAE. However the relative contribution of distortion and reflection in DPOAE generation depends on the stimulus parameters used, and particularly the f_2/f_1 ratio and stimulus level. Knight and Kemp (2000) used the IFFT method to investigate the relative contributions of the two components at different stimulus levels and frequency ratios. They recorded DPOAE across a wide range of stimulus, frequency and frequency ratio parameters, and examined the phase data. They showed in humans that, at frequency ratios above 1.1, the DPOAE is dominated by short latency emission: the distortion component. At smaller frequency ratios, the main component is a long latency emission: the reflection source. The increasing role of the place fixed reflection component with reducing frequency ratio is thought to be due to a reduction in suppression of the CA at the place of f_{dp} by the primaries. This is substantiated by results that obtained the best correlation between TEOAE and DPOAE results when a small DPOAE frequency ratio was used with low stimulus levels (Knight and Kemp, 1999).

The exact relationship of the reflection and distortion components of DPOAE with stimulus level is unknown. In the same way as TEOAE generation, the reflection component is thought to be negligible at high stimulus levels due to saturation, with DPOAE dominated by the distortion component. With low-level stimulation, reflection is thought to dominate (Konrad-Martin et al, 2001). The relationship with level as a function of frequency is unknown.

2.2.3.4 DPOAE fine structure

DPOAE fine structure arises from fine structure in the reflection component itself, as for TEOAE (see above). Additionally there are phase interactions of the reflection and distortion waves, which interfere in the cochlea or ear canal. A combination of these processes results in the characteristic peaks and troughs of level with frequency (Talmadge et al, 1999). Fine structure can only occur for place-fixed processes, where there is a rapid alteration of phase with frequency. The rapidly changing phase of the reflection component determines the frequency spacing of the fine structure.

The reflection component origin of fine structure was demonstrated experimentally by the addition of a third-tone whilst recording DPOAE. The third tone was used to suppress the frequency of the reflection component during DPOAE production. Suppression of this component removed the DPOAE fine structure (Kummer et al, 1995).

2.2.3.5 Cochlear amplification and DPOAE

DPOAE generation in humans arises from both nonlinearities in OHC function (the cochlear amplification mechanism) that generate distortion, and also from coherent reflection – the same reflection mechanism responsible for TEOAE. Whereas for TEOAE the role of the CA is thought to be important mainly at the reflection site and involves generation of a tall and broad TW, the role of the CA in DPOAE generation is potentially more complex; there are two sites to consider and also two different mechanisms that each may have different relationships with CA function. The cochlear nonlinearities that generate the intermodulation distortion arise from the cochlear amplification processes and also the nonlinearities of the passive cochlea. Changes in CA function are likely to be linked to changes in the nonlinear physiological processes of the OHC, but the exact relationship is unknown. Also, changes in CA function will have little effect on passive nonlinearities within the cochlea. It is probable that changes in the CA will affect the reflection component of DPOAE in the same way as TEOAE.

From studies of DPOAE and HTL, it appears that DPOAE continue to be generated with the CA operating at gain levels insufficient for TEOAE production. At low CA gain there may still be sufficient nonlinearity for the distortion component, even if insufficient for reflection. There is also the passive nonlinearity of the cochlea, unrelated to the CA that allows DPOAE to continue to be produced with an effective CA gain of zero if high enough stimulus levels are used. These differences in generation are likely to be the reason that DPOAE are still generated at hearing levels where TEOAE are not.

2.2.3.6 Active and passive DPOAE generation

In the animal field of DPOAE research, models of DPOAE generation originally proposed "active" and "passive" DPOAE (Whitehead, 1992). In small mammals, there is little or no contribution to the DPOAE from reflection sources, and these active and passive sites were thought to be associated with two different locations on the BM near to f_2.

Mills (1997) described an active component that is physiologically vulnerable, corresponding best to the f_2 position on the BM and dominating the response at low-level stimuli. A passive component that is insensitive to cochlear dysfunction is located at an area basal to f_2 and corresponds to the shift in the peak of the TW with an increase in stimulus level. This latter component dominates responses to high intensities in the region of f_2. The two components were thought to summate to generate the DPOAE that is recorded in the ear canal.

This two-component model of DPOAE in animals has been recently questioned. An experiment by Mom et al (2001) aimed to investigate the origin of high intensity DPOAE; they hypothesised that high intensity DPOAE were physiologically vulnerable. They induced ischaemia in two groups of gerbils: those previously exposed to a high-intensity pure tone at a narrow frequency band to induce a mild hearing loss (exposed group) and those with no exposure to loud sound (non-exposed group). Gerbils from the non-exposed group showed a decrease in DPOAE level at the mid-intensity levels, and little reduction at the high-intensity levels. DPOAE input-output (I/O) function (a graph of DPOAE level versus stimulus level) slope increased from 0.6 dB/dB pre-ischaemia to an average of 2.5 dB/dB in the ischaemic condition. However in the exposed group, ischaemia had a different effect on DPOAE. At the frequencies below the frequency of the exposure tone, the change in DPOAE level was similar to the non-exposed group. However at the frequency of exposure, DPOAE levels generated from both mid and high intensity stimuli were reduced. Mom et al concluded that high-intensity DPOAE were physiologically vulnerable and that this result was not consistent with the two-source model of DPOAE generation. The reduction in level of high-intensity DPOAE depended on the mechanism by which the cochlea was disrupted. BM studies have shown that while ischaemia affects the feedback loop of the OHC force generation mechanism at low intensity stimulus levels, at high levels the difference between a normal and pathological BM is negligible, possibly because the MET channels are still intact. However induction of a threshold shift prior to ischaemia may disrupt the MET processes, making all stimulus levels of DPOAE vulnerable to ischaemia.

Mills (2002) made a detailed study of the notches in gerbil DPOAE recordings across a wide range of parameters. Notches in gerbil DPOAE were thought to arise from phase cancellation between the passive and active components and to provide evidence for the active/passive two-source generation mechanism of DPOAE in small mammals (Mills, 1997). Mills (2002) measured contour maps of DPOAE level (a plot of constant DPOAE level against varying stimulus level). Within the contour maps he observed two types of consistent notches, named L1 and L2 notches. L1 notches occurred when L1 was greater than L2, and always occurred at the same L1 value and place on the contour map. There was a rapid change in phase associated with the notch. When L1 was equal to or less than L2 the notch disappeared. The L2 notch occurred when L2 was greater than L1 and similarly was always measured at the same L2 level.

Mills concluded that the presence of these notches at constant L1 or L2 stimulus levels (independent of L2 or L1 respectively) was not explained by phase cancellation of active and passive components. The fact that the notches always occurred at exactly the same stimulus level was likely to be related to the cochlear response to the stimulus. The notch was thought to be associated with the change in the shape of the travelling wave from sharply peaked to broad and rounded as the stimulus level increased. At these constant L1 or L2 levels, the amplitudes of the components results in phase

cancellation, leading to the notch in the I/O function. Mills (2002) concluded that only one mechanism was contributing to DPOAE generation in gerbils.

2.2.4 Comparison of DPOAE and TEOAE

The relationship between OAE and HTL is dependent on the type of OAE measured. The relationship between TE and DPOAE will depend on the dominant component of each OAE type, which is likely to depend on the stimulus parameters used.

TEOAE and DPOAE differ in many ways. The main differences, as summarised by Knight and Kemp (1999) are the place of stimulation along the BM, steady stimulation versus post-stimulation and sequential versus simultaneous sampling of frequencies. Whether DP or TEOAE bear more resemblance to auditory status remains to be investigated.

Available models do not predict the relative effects of changing HTL on DP and TEOAE. Shera and Guinan (1999) suggest that TEOAE will give the best measure of CA function and will be more sensitive than DPOAE. They propose that the organisation of reflected wavelets in TEOAE generation depends on the CA near the peak of the TW for any particular frequency. Reduction in CA gain will reduce this and hence the level of the TEOAE (presumably absence of OHC due to cochlear damage may also reduce the potential reflection sites). They also state that a reduction in gain will have less effect on DPOAE because there may still be sufficient nonlinearity to generate intermodulation distortion. For these reasons, they predict that salicylate will have a greater effect on TEOAE than DPOAE.

2.3 THE RELATIONSHIP BETWEEN OTOACOUSTIC EMISSIONS AND HEARING THRESHOLD LEVEL

The necessity of cochlear amplification for acute auditory sensitivity and the influence of the cochlear amplifier on OAE generation suggest that OAE and HTL may be closely related and that OAE may be used as an alternative to HTL for investigating cochlear function.

The following section describes studies using OAE to investigate HTL. The relationship between OAE and HTL is described, and a review of experimental and clinical models investigating the relationship between OAE and HTL in human subjects is given.

2.3.1 TEOAE

2.3.1.1 TEOAE level

Much research has been carried out examining the relationship between TEOAE level and HTL. Many studies have investigated parameters to find the best correlation between TEOAE level and HTL. The literature describes two main types of experiment: cross-sectional investigating differences in TEOAE level between subjects with differing HTL, and longitudinal examining changes in TEOAE level within subjects with changing HTL. Both these will be discussed.

Cross-sectional studies

Studies looking at differences in TEOAE level between subjects with different HTL have shown a general relationship of decreasing emission level with increasing HTL. However the between-subject variability is high and also frequency dependent (Kemp et al, 1986; Gorga et al, 1993b; Prieve et al, 1993). At 500 Hz, the correlation of HTL with TEOAE level is low, but increases at 2 and 4 kHz. At 4 kHz, the relationship of TEOAE level with behavioural threshold is the most clearly defined, but the spread of values among subjects is still wide. The range of TEOAE level values within normal hearing subjects is approximately –10 to 20 dB SPL (Gorga et al, 1993b; Ferguson et al, 2000). Collet et al (1991) using a multiple regression analysis demonstrated a correlation between the audiogram and the power spectrum of the TEOAE. Large level emissions at the high frequencies were consistent with lower behavioural thresholds at these frequencies. However this relationship was complex and involved other frequency bands and audiometric frequencies. They concluded that hearing loss could not be predicted accurately from emission level. Suckfüll et al (1996) reported a low correlation between TEOAE level and audiometric threshold. The best fit was obtained at 1 kHz but was associated with wide variability of approximately 20 to 30 dB.

The experiments described above all used similar parameter settings and TEOAE were evoked using click levels of approximately 80 dB SPL and the nonlinear derived subtraction method (in which

linear components of the TEOAE are subtracted, leaving only the nonlinear components). This could account for the poor correlation between TEOAE level and HTL, as the reflection-source generation model suggests that TEOAE obtained with lower evoking stimulus levels are more sensitive to differences in cochlear function between subjects. Marshall and Heller (1996) measured TEOAE for click stimuli at intensities of 62, 68, 74 and 82 dB SPL. They found the correlation between TEOAE and HTL was improved when lower stimulus levels were used and there was no associated loss of reliability. Hatzopoulos et al (1995) evaluated the sensitivity of a new classification algorithm at four click levels, 50, 62, 68 and 80 dB SPL, to detect hearing loss. They found levels of 68 and 80 dB SPL were the most sensitive for identifying cochlear hearing loss.

Other studies have used a different methodology. These have compared groups of normal hearing subjects where one of the groups with normal hearing has been exposed to an ototoxic agent (e.g., noise; Desai et al, 1999) or has a disease known to affect the cochlea (e.g., chronic renal failure; Samir et al, 1998). Lucertini et al (2002) compared TEOAE level in three groups of ears: those with bilateral normal hearing, the normal hearing ears of those with unilateral hearing loss and the impaired ears of those with unilateral hearing loss. They showed significant differences between the groups, with the largest TEOAE level measured in the first group; this progressively decreased through group 2 and group 3. Lucertini et al (2002) concluded that TEOAE was measuring subclinical hearing damage in group 2. However hearing threshold was not measured accurately in this experiment – normal hearing was classified by screening audiometry (≤20 dB). For ears that failed screening audiometry, hearing threshold was then measured in 5 dB steps.

All these studies comparing TEOAE in normal hearing subjects and normal hearing, at-risk subjects have shown reduced level TEOAE in the at-risk group compared to the controls, even though both have normal hearing. The general conclusions that have been made from these studies are that TEOAE are sensitive to cochlear dysfunction, and that they detect these changes before they result in changes in HTL. These suggest that TEOAE are measuring a change in cochlear function that does not result in a direct change in hearing sensitivity. These may be minor changes in OHC function that do not affect HTL, but result in a reduction in reflection capability.

However the results can also be interpreted another way, taking into account the mechanisms of TEOAE generation. Avan et al (1997) showed that hearing loss outside the frequency range of the TEOAE spectrum gave a reduction in TEOAE level. The studies described previously only measured hearing at the conventional audiometric frequencies, and did not measure at intermediate or extra high frequencies. Ototoxic drugs are known to affect the high frequencies, and difference in TEOAE level between normal and at-risk groups could be explained by differences in high-frequency hearing (i.e., the differences in level were due to differences in hearing threshold at frequencies not measured in the experiment).

Table 2-2 summarises the results of some of these studies to allow comparison of the correlation coefficient values relating differences in TEOAE with differences in HTL (only studies that provide correlation coefficients values are included in the table). The highest correlations occur at the mid-frequencies of 1 and 2 kHz. TEOAE contain most energy at these frequencies (Kemp et al, 1986) and this spectral range may be most sensitive to changes in hearing threshold. This is not entirely consistent with results from hearing screening experiments, which show TEOAE is best at separating hearing from hearing impaired subjects at audiometric frequencies of 2 and 4 kHz (Gorga et al, 1993b).

The correlation coefficients relating TEOAE level and HTL are generally low and show that TEOAE level explains up to a maximum of 55% of the variation in HTL between subjects. This can be interpreted in either of two ways. Firstly that TEOAE level is a poor indicator of auditory sensitivity, and does not reflect differences in hearing between subjects. Alternatively it may be that TEOAE level is a good indicator of hearing threshold, but other factors (outside the cochlea) are also contributing to variation in level and are masking the relationship between TEOAE and HTL. These factors could include differences between subjects such as external and middle ear anatomy and impedance.

The evidence suggests that TEOAE evoked by lower stimulus levels are more sensitive to differences in HTL. Further investigations using low stimulus levels may improve the correlation between HTL and TEOAE. Even at intensity levels down to 50 dB SPL, TEOAE have long-term reproducibility greater than 75% (Antonelli and Grandori, 1986). However there are practical problems with recording at lower levels. These include difficulty detecting the emission above the noise floor, particularly at the higher frequency bands and if there is a hearing impairment (Marshall and Heller, 1996). The response at low levels may also become dominated by the frequency components of any spontaneous activity (Kemp et al, 1990), which may become synchronised to the click train.

Longitudinal studies

Longitudinal studies of changes in OAE and HTL within-subjects offer advantages over cross-sectional studies. Longitudinal studies are able to control for inter-subject and inter-ear factors that may affect OAE and HTL in different ways, so masking any underlying relationship. Such longitudinal studies have high statistical power and effectively cancel out anatomical differences between subjects as they compare changes within subjects. If TEOAE level is a good indicator of HTL, it may be that changes in TEOAE level and changes in HTL within subjects have a higher correlation than differences in TEOAE level and HTL between subjects.

Studies looking at changes within subjects have generally induced temporary hearing threshold shift and TEOAE through noise exposure. Hotz et al (1993) measured TEOAE in two groups of soldiers before and after 15 weeks of basic training, which included firearms training. All soldiers had normal

hearing prior to entering military service and those in group I were subjected to less noise than those in group II. Following basic training, both groups showed a significant reduction in overall emission level in the left ears and a significant reduction at the 2-4 kHz frequency region in both ears. Group II showed a larger reduction in level at 2-4 kHz than group I. No significant reduction in level was found at lower frequencies. Noise affects the higher frequencies, and these results are consistent with damage due to noise exposure. No data for pre- and post-audiometry are available, so the authors were unable to correlate emission level reduction with changes to pure tone thresholds. The parameter settings used to evoke TEOAE were not described and are assumed to be the default settings of the ILO288 machine used: nonlinear derived subtraction method, 80 dB SPL click.

Another study that compared TEOAE before and after military service was carried out by Engdahl et al (1996). TEOAE evoked by 80 dB SPL click stimuli were obtained from 61 military recruits before and after two months of military training. Following military training, there was a significant reduction in the broadband TEOAE level and also at the 2, 3 and 4 kHz frequency regions of the TEOAE on the left ears and at 1 and 3 kHz on the right ears. These left and right ear level differences were similar to the results of Hotz et al (1993). A reduction in TEOAE level in soldiers following military noise exposure has also been reported by Plinkert et al (1995). The changes occurred mainly at the 0.5-2 kHz region of the TEOAE. However pre-exposure TEOAE level levels were recovered after 15 minutes. These studies did not examine the relationship between the change in TEOAE level and the change in HTL.

Kvaerner et al (1995) looked at the effect of noise on the TEOAE level of employees in an iron works exposed to 7 hours noise daily. Transient emissions were obtained for an 80 dB SPL click on three consecutive days before and after the shift. They found a significant difference in the median TEOAE level following noise exposure compared to pre-exposure. The median level reduction was 0.65 dB. There was no significant reduction in the 3-6 kHz frequency band of the TEOAE. There was also no correlation between the group TEOAE level reduction and the temporary threshold shift (TTS) at either 4 or 6 kHz.

Marshall and Heller (1998) investigated the relationship between TTS and TEOAE level in 14 volunteer subjects. TTS was induced by exposure to 10 minutes of ½ octave band noise centred at a frequency of 1.414 kHz at a level of 105 dB SPL. TEOAE were evoked using a stimulus level of 74 dB SPL and analysed at 2 kHz. Subjects were monitored over six sessions: two pre-exposure, two exposure and one post-exposure session. Their results showed an excellent correlation between the change in TEOAE level and the change in hearing threshold. Also the recovery of the TTS and TEOAE level showed similar functions, although the TTS was approximately 2.5 times greater in amplitude than the change in TEOAE. They concluded that the size of the TTS could be predicted accurately from the change in TEOAE.

Sliwinska-Kowalska et al (1999) measured the change in TEOAE level and the TTS in workers at a factory exposed to noise levels of 85-97 dB(A) for 6 hours. They showed a reduction in TEOAE level and significant TTS, but no significant correlation between the overall group change in TEOAE level and the TTS.

TEOAE level reduction was measured by Liebel et al (1996) in visitors attending a disco, where the noise levels were on average 105 dB(A) and sufficient to induce a TTS. They showed a significant reduction in the TEOAE level at 2 and 3 kHz. However not all subjects showed a reduction in TEOAE level, even some who experienced a TTS up to 15 dB.

Other studies of within-subject changes have measured TEOAE and HTL in subjects taking ototoxic drugs. Yardley et al (1998) measured the change in TEOAE level and HTL in subjects undergoing cisplatin treatment for cancer. Cisplatin is an ototoxic drug known to affect the OHC. They analysed the group change in TEOAE level between the pre-cisplatin and peri-cisplatin conditions. They showed a reduction in TEOAE level that was associated with an increase in HTL. The most significant correlations were between high frequency TEOAE level and HTL at frequencies 2-8 kHz, but there was wide variability.

Table 2-3 summarises the results of these studies to allow comparison of the correlation coefficient values relating changes in TEOAE to changes in HTL (only studies that provide correlation coefficient values are included in the table). These studies of noise exposure and ototoxic drugs have shown a significant effect on TEOAE level. It is accepted that noise primarily affects the OHC of the cochlea, which are known to be the site of the CA. TEOAE are therefore sensitive to OHC dysfunction. However the studies investigating the relationship between TEOAE and a change in HTL seem to be contradictory. Whereas Marshall and Heller (1998) showed a highly significant group relationship between TTS and the change in TEOAE, other studies have not shown similar results. Nonetheless, the design of the studies discussed above meant that although the changes were measured within individuals, the data were all analysed across the group. There have been few studies examining long-term changes in TEOAE and HTL at several time points within individuals, without combining results across subjects. Also most studies used relatively high click levels, and interestingly, the study that showed a highly significant relationship used TEOAE evoked by a lower click level than the others. Further studies employing lower stimulus levels may improve the correlation coefficients reported so far.

The studies of within-subject changes have indicated that TEOAE level is probably sensitive to changes in cochlear function. Most studies did not correlate the change in level with the change in HTL. Most studies compared only pre- and post-exposure changes in level and HTL, rather than obtaining serial measures over time. These studies showed that changes in TEOAE level with changes in HTL are variable between subjects.

Table 2-2: Summary of studies investigating the cross-sectional relationship between TEOAE and HTL

TEOAE measure	Study	Number of ears	Range of HTL (dB)	Click level (dB SPL)	Emission frequency band (kHz)	HTL frequency (kHz)	Correlation coefficient (R)	R-square
Level	Collet et al (1991)	150		80	2.9	4	–0.48	0.23
	Sückfull et al (1996)	118	0 to 60 (estimate)	83	1	1	–0.65	0.42
	Marshall and Heller (1996)	25	–10 to 40	82	Broadband	Mean 1-4	–0.58	0.33
		25	–10 to 40	74	Broadband	Mean 1-4	–0.65	0.42
		25	–10 to 40	68	Broadband	Mean 1-4	–0.66	0.43
		25	–10 to 40	62	Broadband	Mean 1-4	–0.65	0.42
Detection threshold	Bonfils et al (1988)	240	–5 to 35	-	Broadband	Click	0.74	0.55
		240	–5 to 35	-	Broadband	Mean 1–4	0.53	0.29
	Tanaka et al (1990)	15	10 to 60	-	Broadband	Mean 0.5-4	0.85	0.72
	Avan et al (1991)	160		-	1	2		
	Avan et al (1993)	124	–10 to 80	-	1	2	0.77	0.60

2.3.1.2 TEOAE detection threshold

TEOAE detection threshold has been proposed as an alternative measure of HTL. The detection threshold is the lowest evoking stimulus level at which TEOAE are generated. This is thought to have similarities with hearing threshold. However a disadvantage of this method is that TEOAE detection threshold is also a property of the TEOAE measuring equipment as well as a physiological property of the ear. It depends heavily on the sensitivity of the probe microphone and the noise floor of the measuring equipment.

Cross-sectional studies

Studies have shown a general relationship between emission threshold and psychoacoustic threshold, with emission threshold increasing with increasing hearing threshold up to a mean HTL level of 35 dB HL at which point TEOAE are not recorded (Bonfils et al, 1988b). The range of TEOAE threshold between subjects is wide, and spans approximately 30 dB in normal hearing subjects (Avan et al, 1991). Avan et al, (1991, 1993) showed a significant correlation between the 1 kHz TEOAE threshold and the pure tone threshold at 2 kHz. However this relationship was found to be independent of the site of hearing damage. See Table 2-2 for a summary of the cross-sectional studies.

Longitudinal studies

Berninger et al (1998) investigated the relationship between the change in pure tone threshold and TEOAE detection threshold in subjects taking quinine. They tested six subjects who were given sufficient quinine to induce a TTS. The change in TEOAE and HTL was monitored over a 32 hour period. They showed a significant linear relationship between the increase in TEOAE detection threshold and the increase in psychoacoustic threshold to the TEOAE click stimulus over quinine administration at 1 and 2 kHz. The relationship between the two variables was approximately 1 dB/dB.

Table 2-3 summarises the results of longitudinal studies relating TEOAE detection threshold and HTL. The study by Berninger et al (1998) shows that changes in detection threshold within subjects appear to have a higher correlation with HTL than the differences between subjects. Even though this study was only based on 6 subjects, it provides evidence that unrelated differences between subjects may be responsible for the low correlation coefficients of the cross-sectional studies.

Table 2-3: Summary of studies investigating the longitudinal relationship between TEOAE and HTL

TEOAE measure	Study	No of ears	Click level (dB SPL)	OAE frequency band (kHz)	Mean (range) OAE shift (dB)	HTL frequency (kHz)	Mean (range) HTL shift (dB)	Correlation coefficient (R)	R-square
Level	Kvaerner et al (1995)								
	Marshall and Heller (1998)	17	74	2	4.7	2	12	0.98	0.96
	Sliwinska-Kowalska et al (1999)	62	80	Broadband	1.2 (–1.2 to 3.7)	6	9 (–5 to 32)	0.1	0.01
		62	80	4	2.3 (–5.6 to 8.7)	6	9 (–5 to 32)	0.2	0.04
Detection threshold	Berninger et al (1998)	12	-	Broadband	Not given (–2 to 16)	Click stimulus	9.7 (2 to 18)	0.88	0.77

2.3.1.3 TEOAE periodicity

Avan et al (2000) tested a model relating periodicity of the TEOAE spectrum with cochlear fine-tuning. Cochlear fine tuning is associated with normal cochlear amplification. The theory of coherent reflection filtering predicts that TEOAE spectrum periodicity relies on 'tall and broad' cochlear filter shape. Fine-tuning is thought to be associated with close frequency spacing of the spectral peaks of the TEOAE, and normal ears typically have spectral periods of 0.4 Bark. The model predicts that a decrease in cochlear tuning will lead to an increase in the width of the 'tall and broad' filter, giving an increase in the frequency spacing of the TEOAE spectrum. Avan et al (2000) compared the periodicity of TEOAE spectrum from two groups of subjects: those with normal hearing and those with noise-induced hearing loss (NIHL). TEOAE were obtained using a stimulus level of 70 dB SPL. Although the results were not entirely conclusive, subjects with NIHL showed less regularity in the TEOAE spectrum compared to the normal hearing group. It is possible that using a lower stimulus level to generate TEOAE may have generated more conclusive results. A stimulus level of 70 dB is relatively high, and TEOAE generation at this level may not have been dominated entirely by reflection processes and may have involved contributions from many filters thorough distortion processes. Using a lower stimulus level, which is thought to reveal more about the reflection mechanisms, may have shown greater differences between the two subject groups.

2.3.1.4 Maximum length sequence TEOAE

In conventional recording, the maximum click rate that can be used is limited by the length of the TEOAE response. The length of the TEOAE response is approximately 20 ms, therefore the maximum click rate that can be used is 50 clicks/s. If rates greater than 50 clicks/s are used, the TEOAE responses overlap and the overall response becomes contaminated.

Maximum length sequences (MLS) are a way of increasing the stimulation rate whilst preserving the integrity of the response, thus giving the advantage of reduced recording time or allowing more sweeps to be collected for the same recording time. They allow the use of stimulus rates where the time between successive stimuli is less than the length of the response. Thornton (1993a, b) was the first to record TEOAE using MLS. Since then, MLS TEOAE has been proposed as an improved method over conventional recording techniques for neonatal hearing screening due to advantages of improved signal-to-noise ratio (SNR) and a decreased test time (Thornton, 1993a, b; Rasmussen et al, 1998).

MLS in an audiological application is a quasi-random binary sequence of clicks and silences represented mathematically by 1 and 0 respectively (Thornton et al, 1994). The length (L) of an MLS (or the total number of clicks and silences) is $2^{order} - 1$.

Of the MLS, (L+1)/2 elements (represented by ones) trigger the stimulus, the rest are silences. The clicks and silences within the MLS length are known as "click opportunities". When discussing MLS

rate, the most common terminology is maximum rate, which is defined as the reciprocal of the minimum interstimulus interval (the time difference between two click opportunities). Maximum rate has the closest resemblance to the rate used in conventional recording. There is no change in maximum rate with a change in MLS order. The choice of MLS order is determined by a number of factors including the length of the OAE time window. The duration of the MLS sequence must be greater than or equal to the time window otherwise aliasing errors can occur.

The response can be deconvolved using a recovery sequence of the same length and sequence as the MLS. For more detailed information on TEOAE deconvolution, refer to Thornton et al (1994). This deconvolution is generally performed in real time, allowing time domain averaging during the recording process. This is necessary to employ effective noise rejection criteria, which is important for any clinical recording. Each sample is "recovered" as it is collected allowing each recovered response to be checked against a rejection criterion whilst recording is ongoing. As the last response is recovered, it is added to its place in the sequence to give the recorded TEOAE.

There is a reduction in TEOAE level with increasing click rate, which has been well documented (Hine and Thornton, 1997). TEOAE rate suppression is defined as the difference in level between TEOAE generated at a reference click rate (e.g. 50 clicks/s) and TEOAE generated at a higher click rate (e.g. 10,000 clicks/s). It has been proposed that rate suppression arises from the same cochlear properties responsible for the TEOAE I/O level-intensity function (Picton et al, 1993; Hine and Thornton 1997). Hine and Thornton (1997) showed a significant relationship between the TEOAE level at 40 clicks/s and the rate effect (R-square = 0.99). Rasmussen et al (1998) correlated the rate effect with the slope of the I/O function in a group of normally hearing subjects. They measured rate suppression over three different ranges of rates (500-2000, 250-2000 and 125-2000 clicks/s). This gave median correlation coefficients of 0.59, 0.66 and 0.77 respectively.

The evidence therefore suggests that rate suppression is of cochlear origin rather than an effect of the efferent system. More specifically it is thought that the same processes responsible for the nonlinearity of the I/O function are responsible for the rate suppression effect. The nonlinearity of the I/O function arises from the properties of the cochlear amplifier, and reduction in CA function is considered to give a reduction in nonlinearity. This is substantiated by studies examining the relationship between TEOAE I/O functions and hearing loss, which have shown an increase in the slope of the I/O function with increasing hearing loss.

Kapadia and Lutman (2001) proposed a simple model relating TEOAE rate suppression to the compressive nonlinearity of the TEOAE I/O function, where the nonlinearity of the I/O function is described using the equation for a straight line, $y = Ax^m$. With increasing rate, the TW responses on the BM show increasing overlap. This results in greater level of vibration of the BM, resulting in greater saturation of the cochlear amplifier. This effect of increasing rate on the BM is thought to be

similar to the effect of increasing stimulus level. This model assumes a single channel model of TEOAE generation, rather than distributed sources generation, with no interaction between channels. The model was tested by computer simulation, using a 1 kHz Hanning windowed tone burst input. Maximum lengths sequences of the input were applied to the model, and the results of the MLS were compared to a single input, conventional stimulus.

A linear system (m=1) showed no difference in level output between the MLS and the conventional stimuli. A nonlinear system (m<1) showed a reduction in output as the MLS rate was increased. Increasing the nonlinearity of the system (by reducing m) also resulted in a decrease in output. Kapadia and Lutman (2001) proposed that the results of the model could be applied to cochlear TEOAE I/O functions, where the lower the slope of the I/O function (i.e. greater compressive nonlinearity) the greater the level of rate suppression (see Figure 2-5). They argued that this model supports a cochlear basis for rate suppression and that rate suppression is directly related to cochlear nonlinearity. The model predicted a reduction in TEOAE level of 6 dB in a system with an I/O function slope of 0.5 dB/dB, at a click rate of 5000 clicks/s.

This model of cochlear nonlinearity could be used as an indirect estimate of HTL. It is arguable that subjects with low HTL have highly compressive nonlinear I/O functions and thus show a large rate suppression effect. As HTL is increased, the I/O function becomes less compressive and the rate suppression effect is reduced. This model has not been fully tested in human subjects and requires further investigation. It requires validation in subjects with a range of hearing losses, and also in subjects undergoing hearing loss or exposed to ototoxic drugs.

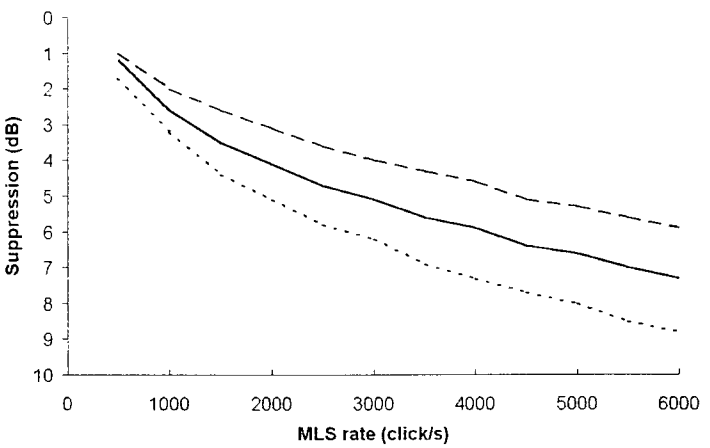


Figure 2-5 Results derived from the MLS TEOAE rate suppression model. Each line represents different slope values of the I/O function. Key – dashed line: m=0.6, solid line: m=0.5, dotted line: m=0.4 (adapted from Kapadia and Lutman, 2001).

2.3.1.5 Gaps in knowledge

There are several different TEOAE measures that are likely to adequately reflect HTL. Of the cross-sectional studies all TEOAE measures reported have similar relationships with HTL and correlation values ranged from 0.2 to 0.7. The mid HTL and TEOAE frequencies show the highest correlations.

Many of the experiments have been limited by their use of high stimulus levels to evoke TEOAE even though recent evidence suggests lower level stimulus levels may be more sensitive to HTL. Also many of the experiments have not made careful measurements of hearing.

Most of the experiments have been cross-sectional, and there have been relatively few longitudinal experiments. The between-subject variability is high, and it is likely that within-subject experiments will show better relationships between TEOAE and HTL. There is therefore a need for longitudinal studies, investigating more complex models of TEOAE to determine whether measures such as TEOAE rate suppression have a higher correlation with HTL than TEOAE level. These should use lower stimulus levels and recording equipment that allow TEOAE to be measured to lower levels and to a lower noise floor.

2.3.2 DPOAE

2.3.2.1 DPOAE level

In the same way as studies of TEOAE explored level as an indicator of HTL, DPOAE level has also been investigated. Both cross-sectional and longitudinal studies are described here in relation to the distortion product $2f_1-f_2$. Only limited data are available on other DPOAE components and they are disregarded in the following sections. In most studies, the f_2/f_1 frequency ratio was set to produce the maximum DPOAE, typically using a ratio close to 1.22. Only limited data are available for other frequency ratios.

Cross-sectional studies

There is a general relationship of decreasing DPOAE level with increasing HTL. However the correlation varies across the frequency range. At frequencies of 4, 6 and 8 kHz the correlation is highest, although variability between subjects is high (Harris and Probst, 1991; Gorga et al, 1993b; Gorga et al, 1996; Gorga et al, 1997). At the lower frequencies, there is no significant relationship between the two variables. Probst and Hauser (1990) quantified the relationship between HTL and DPOAE level. In their correlation analysis, they included frequencies across the range, and obtained a low correlation. This is likely to be a result of including low frequency DPOAE in the analysis. Arnold et al (1999) performed an experiment to assess the influence of extra high frequency (EHF) hearing thresholds on lower frequency DPOAE. They compared two groups of subjects: one group had normal hearing at the conventional audiometric frequencies and also at EHF of 11.2 to 20 kHz. The other group had normal hearing at the conventional audiometric frequencies but worse hearing at

the EHF. The group with poorer hearing at the EHF had reduced DPOAE level at 4 to 8 kHz. This suggests that changes in function at the basal end of the cochlea may affect recording of more apical DPOAE. This may account for some of the variability in the relationship between DPOAE and HTL.

Table 2-4 summarises the results of recent studies that have examined the relationship of DPOAE level and audiometric threshold. At the high frequencies, DPOAE level explains approximately 60-70% of the variation in HTL.

These studies all used mid-intensity stimulus levels but the use of lower stimulus levels may have improved the correlation values provided the noise floor of the measurement system was low enough. They all show a fairly high correlation between DPOAE level and HTL in the expected direction, but with wide variability between subjects. However the correlation is higher than the correlation between TEOAE level and HTL. The implication is that DPOAE level is related to HTL, but that variation due to other between-subject differences may be reducing the correlation. DPOAE fine structure may also be contributing to the poor relationships, in that measurement of DPOAE level may by chance fall at a peak or a trough within the fine structure, varying across subjects.

Longitudinal studies

DPOAE are sensitive to noise exposure, with maximum changes in DPOAE level occurring at ½ octave above the frequency of the noise. Sutton et al (1994) exposed human subjects to a 105 dB SPL 2.8 kHz tone for 3 minutes and measured DPOAE level at 4 kHz pre- and post-exposure. They compared the sensitivity of four L1/L2 stimulus paradigms, 60/60, 60/30, 55/55 and 55/30 dB SPL for detecting changes in DPOAE level post-exposure. Following noise exposure there was an initial significant downward shift in baseline level of the DPOAE, which then recovered to pre-exposure levels. When L1 and L2 were set at unequal levels, the change in DPOAE level was greater than when at equal levels. The greatest change occurred at the lowest intensity level 55/30. Although this study only compared four different levels of L1 and L2 at one frequency, this would suggest that unequal levels of L1 and L2 are more sensitive to detecting changes due to noise. The relationship of the change in DPOAE with TTS is not reported.

Engdahl (1996) investigated the effects of noise and exercise on DPOAE level. Eight normally hearing subjects were exposed to 102 dB SPL third octave band noise with a centre frequency of 2 kHz. DPOAE level was measured between 2 to 4 kHz using 55/40 dB SPL primary tones. The change in DPOAE level was correlated to the noise-induced TTS. At 1 minute post-exposure, there was no significant relationship between the change in DPOAE and the change in hearing. However removal of two outliers gave a weakly significant relationship between the two variables.

Engdahl and Kemp (1996) exposed subjects to 102 dB SPL 2 kHz tone for 10 minutes. DPOAE level measured at 3 kHz using 55/40 dB SPL primaries gave similar results to those of Sutton and colleagues with the largest level reduction occurring immediately following noise exposure and then

recovering to pre-exposure levels after 32 minutes in most subjects. The correlation between the degree of DPOAE level reduction and the temporary threshold shift was not described.

Littman et al (1998) described the case of paediatric patient undergoing cisplatin treatment. Three test sessions are described: pre-, peri- and post-cisplatin treatment. At each session they measured DPOAE with f_2 at the octave and intermediate audiometric frequencies, L1/L2 set to 65/50 dB SPL and the f_2/f_1 ratio at 1.22. Pure tone audiometry was also measured at the octave and mid-octave frequencies. Cisplatin treatment caused a bilateral high-frequency sensorineural hearing loss and a reduction in DPOAE level. They correlated the audiometric results with the DPOAE results with same f_2 frequency, and showed DPOAE level was reduced prior to the audiometric results. They do not report the relationship of DPOAE with other pure tone frequencies.

Berninger et al (1995) induced TTS in five subjects using quinine. DPOAE were evoked using equal L1/L2 at 75 dB SPL at f_2 frequencies of 0.7, 1, 1.5, 2, 3 and 4 kHz. The mean change in DPOAE level was 1.4 dB. They did not correlate the change in DPOAE level with the change in HTL.

Berninger and Gustafsson (2000) performed a further experiment to induce TTS in seven subjects using quinine. DPOAE were evoked using a range of stimulus levels, with L1/L2 varied in 5 dB steps from 70 dB SPL to the noise floor. The frequency range was as described in Berninger et al (1995). The largest mean shifts in DPOAE level were measured at the lowest stimulus levels (a mean shift of 10.5 dB at a stimulus level of 40 dB). There was a significant increase in the mean slope of the I/O function (0.86 to 1.35 dB./dB). However there was no significant correlation between the change in DPOAE level and the change in HTL.

Doring et al (1998) investigated the effect of disco music sufficient to generate a TTS, on DPOAE and HTL. DPOAE I/O functions were measured at f_2 equal to 4 kHz. HTL was measured at the audiometric frequencies 1 to 8 kHz in 1 dB steps. DPOAE level was calculated as the area under the regression line of the I/O function, and the change in DPOAE was calculated as the change in the area under the I/O function. Interestingly, from their results some subjects demonstrated changes in DPOAE level that paralleled the change in HTL. However other subjects showed an initial change in DPOAE level with no recovery, and other showed a recovery in DPOAE level back to pre-exposure levels before the end of the noise exposure period. Overall, the group mean results showed a relationship between changes in DPOAE level and HTL, with the change in DPOAE approximately one-third the change in HTL. Correlation coefficients are not reported.

Marshall et al (2002) compared DPOAE in sailors exposed to aircraft noise pre- and post-six month deployment. DPOAE were measured at four stimulus levels. The sailors showed no change in HTL after the exposure, but there was a significant reduction in DPOAE level. There was no significant correlation between the change in HTL and the change in DPOAE.

Table 2-5 summarises the results of these studies to allow comparison of the correlation coefficient values relating changes in DPOAE to changes in HTL (only studies that provide correlation coefficient values are included in the table). Low intensity primary tones have been shown to be more sensitive at detecting temporary changes in DPOAE due to noise in human subjects. Recommended levels of L1/L2 (dB SPL) to maximise sensitivity are 55/40 (Gaskill and Brown, 1990; 1993), 55/30 (Sutton et al, 1994) and 45/35 (Whitehead et al, 1995a,b). Stover et al (1996) also found levels of 65/55 were most effective at separating hearing and hearing impaired ears. This is likely to be due to the distortion and reflection mechanisms responsible for producing DPOAE at high and low level stimuli (Gaskill and Brown, 1990; Zwicker and Harris, 1990; Harris and Probst, 1991). The lowest stimulus level that can be used is influenced by the system noise of the equipment; if this is low enough then lower level primaries can give good quality recordings.

Table 2-4: Summary of studies investigating the cross-sectional relationship between DPOAE and HTL

DPOAE measure	Study	Number of ears	Range of HTL (dB)	f_2/f_1 ratio	L1 (dB SPL)	L2 (dB SPL)	f_2 (kHz)	Pure tone frequency (kHz)	Correlation coefficient (R)	R-square
Detection threshold	Kimberley and Nelson (1989)	53	–5 to 90	1.20	L2	30 to 80	4	4	0.81	0.66
			20 to 90	1.20	L2	30 to 80	4	4	0.41	0.17
	Lonsbury-Martin and Martin (1990)	15	5 to 65		Not stated		3	3	0.85	0.72
	Nelson and Kimberley (1992)	53	0 to 100 (estimate)	1.20	L2	30 to 78	4	4	0.81	0.66
	Avan and Bonfils (1993)	75		1.23	L2	42 to 72				
	Sliwinska-Kowalksa (1998)	240	15 to 60	1.22	L2	25 to 70	4	4	0.47	0.22
	Dorn et al (2001)	27	0 to 90	1.22	0.4L2 + 39	–5 to 95	4	4	0.86	0.74
	Boege and Janssen (2002)	149	–10 to 80	1.20	0.4L2 + 39	20 to 65	0.48 to 8	0.48 to 8	0.65	0.42
Level	Probst and Hauser (1990)	199	–10 to 100	1.15 to 1.35	73	63	1 to 4	1 to 4	0.52	0.27
	Avan and Bonfils (1993)	75		1.23	62	62				
	He and Schmiedt (1996)	38		1.20	50	50		Mean 2 to 6	0.56	0.31
	Kim et al (1996)	135	–10 to 85	1.20	65	65	6	6	0.83	0.69
		142	–5 to 85	1.20	65	65	1	1	0.74	0.55
	Suckfull et al (1996)	102	0 to 60 (estimate)	1.22	70	70	6	6	0.84	0.71
	Sun et al (1996)	75	0 to 90	1.18 to 1.23	65	50	4	4	0.87	0.76
	Sun et al (1996)	77	–5 to 95	1.18 to 1.23	65	65	6	6	0.80	0.64
	Sliwinska-Kowalksa (1998)	240	15 to 60	1.22	70	70	4	4	0.61	0.37

Table 2-4 continued: Summary of studies investigating the cross-sectional relationship between DPOAE and HTL

DPOAE measure	Study	Number of ears	Range of HTL (dB)	f_2/f_1 ratio	L1 (dB SPL)	L2 (dB SPL)	f_2 (kHz)	Pure tone frequency (kHz)	Correlation coefficient R	R-square
Signal to noise ratio	Gorga et al (1993a,b)	180		1.20	65	50	4	4	0.85	0.75
	Gorga et al (1993a,b)	180		1.20	65	50	8	8	0.71	0.50
I/O function slope	Janssen et al (1998)	39	10 to 70	1.20	0.4L2 + 39	40 to 60	0.48 to 8	0.48 to 8	0.63	0.40
	Kummer et al (1998)	15	0 to 60	1.20	53	35	0.48 to 8	0.48 to 8	0.68	046
Suppression tuning curve	Pienkowski and Kunov (2001)	23	−10 to 30	1.20	60	40	4	4	0.67	0.45

Table 2-5: Summary of studies investigating the longitudinal relationship between DPOAE and HTL

DPOAE measure	Study	No of ears	f_2/f_1 ratio	L1 (dB SPL)	L2 (dB SPL)	f_2 (kHz)	Mean (range) OAE shift (dB)	HTL frequency (kHz)	Mean (range) HTL shift (dB)	Correlation coefficient R	R-square
Level	Engdahl (1996)	8	1.22	55	40	Mean 2 to 4	6 (estimate)	3	15	0.24 (including outliers)	0.05
	Engdahl (1996)	6	1.22	55	40	Mean 2 to 4	6 (estimate)	3	15	0.83 (excluding outliers)	0.69
	Berninger and Gustafsson (2000)	6	1.22	50	50	Combined 0.75 to 6	Mean not given (−6 to 28)	Combined 0.75 to 6	Mean not given (5 to 30)	0.2	0.04

2.3.2.2 DP-gram

DPOAE level varies with frequency of the primary tones. This relationship is graphically represented as a DP-gram with DPOAE level plotted against either f_2, f_1 or the geometric mean of the primaries. DP-grams are variable between subjects but stable with time (Gaskill and Brown, 1990). At fine frequency intervals the DP-gram exhibits a fine structure of characteristic peaks and troughs giving a "rippling" effect across frequency, which becomes less marked above 6 kHz (He and Schmiedt, 1993). The peak-peak intervals are spaced at approximately 3/32 octave independent of age or hearing loss (He and Schmiedt, 1993; He and Schmiedt, 1996; Engdahl and Kemp, 1996), whereas the peak-valley depths are more variable and can be as large as 20 dB (He and Schmiedt, 1993), changing at a rate of 250 dB/oct in some cases (Gaskill and Brown, 1990). The peak-valley depth is also known as the maximum to minimum ratio (Engdahl and Kemp, 1996). As cochlear frequency resolution is decreased, the fine structure disappears. Fine structure is also altered by changing the levels of the primaries. As level is increased, the rippling of the DP-gram shifts towards the low frequencies and reduces in magnitude (He and Schmiedt, 1997). Heitmann et al (1996) estimated the mean shift to be 50 Hz for an increase of 15 dB in the primary tones.

Cross-sectional studies

DP-grams can show notches at specific frequencies that are equivalent to areas of hearing loss in the pure tone audiogram (Ohlms et al, 1990) and overall reduction in level when behavioural thresholds lie outside the normal range (Smurzynski et al, 1990). However these studies did not analyse the relationship between the reduction in DPOAE level and the degree of hearing loss. Gaskill and Brown (1990) compared the pure tone audiograms of normal hearing subjects with the respective DP-grams and found a statistically significant correlation in approximately half the subjects. In a later study using hearing and hearing-impaired subjects, they found a correlation between HTL and DPOAE level in one-third of the subjects (Gaskill and Brown, 1993), but in the remainder there was no clear relationship. Suckfüll et al (1996) carried out such a study with hearing and hearing-impaired subjects and plotted a DP-gram across the frequencies from 0.46 to 4 kHz. A correlation analysis relating DPOAE level to hearing threshold showed the highest correlation at the higher frequencies, but variability was still high. Lutman and Deeks (1999) compared DP-grams with audiometry (both measured in 16 Hz steps) in a group of normal hearing subjects. Both the DP-grams and the detailed audiograms showed fine structure, however there was no correspondence between the two.

The relationship between the DP-gram and discrete octave frequencies of the pure tone audiogram has shown a poor correlation. This could be for a number of reasons. Firstly, the frequency spacing of the DP-gram should be small enough to reveal the distortion product fine structure; otherwise the DP frequency might by chance fall at a peak or trough, varying across subjects; Gaskill and Brown (1990) recommend that at least 1/18 octave intervals be used to overcome the large changes in level that can

occur between points on the DP-gram. The same issue affects measurement of HTL, and fine frequency resolution audiometry measured at similar intervals to the DP-gram may slightly improve the correlation.

A study that took a different approach to those described previously was carried out by Mauermann et al (1999). Their study was designed to investigate the two-source generation of DPOAE, and the contribution of the f_{dp} site to DPOAE fine structure. They studied three groups of subjects with specific audiometric configurations: subjects with notches at 4 kHz, those with high frequency hearing loss and those with moderate hearing loss but normal hearing at 1.5 kHz only. DP-grams were measured and the effect of restricting the primary tones or f_{dp} to the normal hearing regions of the cochlea on DPOAE fine structure was studied. Where the f_2 primary tone frequency corresponded to the frequency of hearing loss (but the f_{dp} frequency corresponded to a region of normal hearing), they found a reduction in DPOAE level but no reduction in DPOAE fine structure. However when the f_{dp} frequency corresponded to the region of hearing loss, they found no reduction in DPOAE level but a reduction in fine structure. Their results were generally consistent with the two source generation mechanism, implicating reflection at the f_{dp} site as important in DPOAE fine structure. This shows it is important to take into account both generation sites when investigating the relationship between DPOAE and HTL.

Longitudinal studies

The effect of noise on DPOAE fine structure has been examined (Engdahl and Kemp, 1996). Human subjects were exposed to 10 minutes of narrow band noise centred at 2 kHz at an intensity of 105 dB SPL. DP-grams obtained at a resolution of 28 Hz using L1/L2 at 60/50 dB SPL showed a significant change in level following TTS. The maximum to minimum ratio of the DP-gram was significantly reduced, with the greatest shifts in level occurring at the peaks. There was also a shift of the entire microstructure towards the low frequencies but no change in the peak-peak intervals that might be expected from the models of Zweig and Shera (1993), and Talmadge et al (1999).

2.3.2.3 DPOAE detection threshold

DPOAE level increases with increasing stimulus and is plotted as a DPOAE I/O function. From the I/O function, the detection threshold, dynamic range and growth slope of the emission can all be calculated. The emission threshold is the lowest stimulus level at which the DPOAE can be detected above the noise floor, and this can be derived in a number of ways. The simplest method is to take threshold as the lowest level at which the DP is present at 3 dB above the noise floor (Whitehead et al, 1993); however, this can miscalculate threshold if the I/O function is complex or the noise floor is high. At low signal-to-noise ratios, there is also the problem of the response and the noise summating which artificially increases the response level and in turn alters the slope of the I/O function (Nelson and Zhou, 1996). Nelson and Kimberley (1992) used an algorithm that extrapolates threshold to the

noise floor from the slope of the function. This overcomes the problems of the more complex growth curves, but is still limited to an extent by the noise levels at recording. Whitehead et al (1993) suggested that threshold should be defined as the stimulus level that elicits a DPOAE above an absolute criterion measure thus removing the variability of the noise floor. Alternatively, using time-locked averaging techniques instead of spectral power averaging can lower the noise floor from -10 dB to -30 dB SPL and so allow DPOAE to be recorded to considerably lower levels (Nelson and Zhou, 1996). Typical threshold values in normal hearing subjects range from 0 to 70 dB SPL (Kimberley and Nelson, 1989; Nelson and Kimberley, 1992; Gorga et al, 1996) although this large variation may in part be due to the different recording methods used.

Kimberley and Nelson (1989) investigated the association between HTL and DPOAE detection threshold. All auditory and emission threshold data were combined and they found a high correlation between the two variables. The range of the normal data was large, with detection thresholds ranging from 30 to 70 dB SPL. When the two hearing and hearing impaired groups were separated and the correlation analysis was calculated separately for both groups, the correlation coefficient values were significantly lowered (Nelson and Kimberley, 1992).

Lonsbury-Martin and Martin (1990) examined DPOAE detection threshold and HTL and found the highest correlation at 3 kHz. At this frequency the detection thresholds of the normal hearing subjects ranged from 50 to 60 dB SPL. There was no significant correlation between the DPOAE detection threshold and HTL at 1 kHz.

Gorga et al (1996) examined the relationship between DPOAE detection threshold and HTL. They did not present the correlation coefficient values for the different frequency relationships, but scattergrams showed the closest relationship at the higher frequencies. However there was still wide variability between subjects.

Katbamna et al (1999) compared DPOAE detection thresholds in normal hearing subjects and cystic fibrosis patients with normal hearing undergoing tobramycin treatment. They showed raised detection thresholds in the tobramycin group. Table 2-4 summarises the results of these studies.

2.3.2.4 DPOAE input-output functions

DPOAE I/O functions have been proposed in the literature as a useful measure of OAE to relate to HTL (Dorn et al, 2001). Measuring DPOAE level at differing stimulus levels is thought to measure the CA at different states of saturation. Growth of DPOAE level with stimulus level shows similarities to BM vibration growth with stimulus level: functions from normal hearing ears both show compressive nonlinear functions that saturate at moderate to high intensity levels. From ears with hearing loss, both show a reduction in nonlinear compression.

Although it is based on gerbil ears, Mills (1997) proposed a model relating changes in CA gain to changes in DPOAE I/O functions. It is acknowledged that the model was based on the now outdated concept of an active/passive, two-component model of DPOAE generation in gerbils, however it provides a useful framework. The active component of the I/O function can be thought of the CA at maximum gain, and the passive component can be thought of as the saturated CA. The model predicts a simple relationship between the change in DPOAE level and the change in CA gain. It also makes predictions about the change in the shape of the I/O function with the change in CA gain. For simple cubic distortion, the level of the distortion product has a cubic relationship to the levels of the primaries: hence on decibel scales, the function relating distortion level to primary level has a slope of 3 dB/dB. Therefore, in the absence of gain in the CA, distortion will increase with a slope of 3 dB/dB: this situation is referred to as the linear cochlea (although there must of course be some passive distortion to generate the distortion product). DPOAE from a cochlea with an active CA are predicted by Mills to have nonlinear I/O functions with slope values less than 3 dB/dB as the function is approaching saturation. As CA gain is reduced, the slope of the I/O function increases over the compressive section of the function. See Figure 2-6.

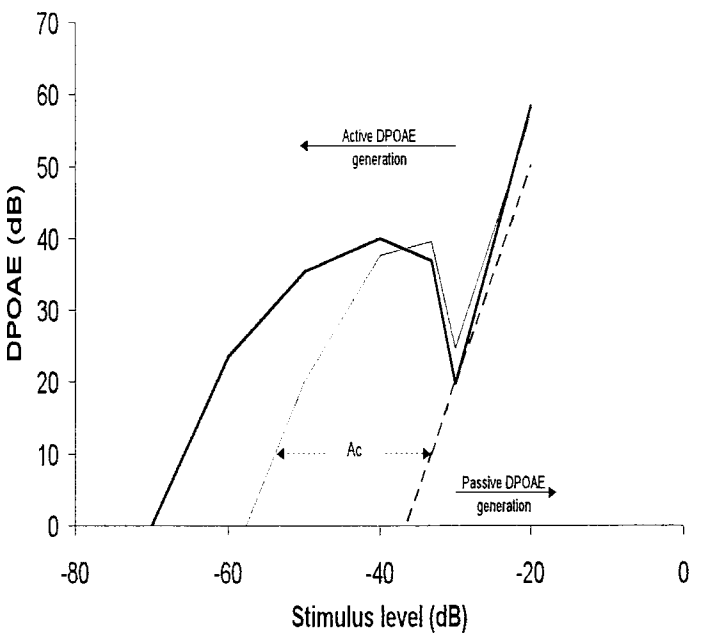


Figure 2-6: Model of DPOAE I/O function and CA gain for gerbil ears. CA gain is represented by Ac. A reduction in CA gain causes a reduction in DPOAE level at the low stimulus levels only. This leads the I/O function to shift to the right of the graph. (Adapted from Mills, 1997).

The growth of DPOAE level with stimulus level varies considerably between individuals (He and Schmiedt, 1993; Whitehead et al, 1995b). Earlier studies showed the relationship to be nonlinear with a high number of non-monotonicities (Harris et al, 1989; Nelson and Kimberley, 1992). Different studies have given quite different results for the slope of the I/O function.

Harris et al (1990) quote slope values of 0.8-1.32 in their group of normal hearing subjects measuring I/O functions from 0.75-6 kHz. Studies have shown an increase in the slope of the I/O functions with an increase in frequency (Harris, 1993; Vinck et al, 1996). I/O functions can be influenced by the noise floor, as described previously. For these reasons, at low stimulus levels the growth rates may have been underestimated. Nelson and Zhou (1996) recorded I/O functions using a time averaging technique and found that the slope of the I/O function was significantly steeper at low stimulus levels than that obtained from spectral averaging with no noise floor correction. This was also steeper than the function obtained at the higher stimulus levels. They postulated that the typical function has two components: a component for primary levels below 40 dB SPL which has a linear growth with stimulus, and a component at moderate and high stimulus levels which is nonlinear, often shows non-monotonicity and reaches saturation about 70 dB. The slope values at 30/40 dB were approximately 1 dB/dB. This should be compared to a theoretical, non-compressive system that would have a growth rate of 3 dB/dB for the distortion product $2f_1-f_2$.

Cross-sectional studies

Most studies of DPOAE I/O functions have examined differences between subjects. These types of studies have shown differences in DPOAE growth with differences in HTL. Harris (1990) measured DPOAE I/O functions across the frequency range in two groups of subjects. One group had normal hearing, and the other group had normal hearing up to 1 kHz and a sensorineural hearing loss at the higher frequencies. The results showed differences in the DPOAE I/O function slope between the two groups. The high frequency hearing loss group had I/O functions with higher slope values (i.e., the DPOAE level growth was more linear than in the normal hearing group). The biggest differences in DPOAE growth were observed at the higher frequencies, but there were still differences at the lower frequencies even though at these frequencies both groups had normal hearing.

A similar experiment was performed by Nieschalk et al (1998). They compared DPOAE I/O functions in two groups of middle-age human subjects. One group had high frequency hearing loss and the other had normal hearing. They showed differences in the slope of the functions between the two groups, even at frequencies where both groups had normal hearing. In the high frequency hearing loss group, I/O functions were steeper and did not plateau at the high stimulus levels.

Nelson and Kimberley (1992) also compared DPOAE I/O functions in two groups of subjects. One group had normal hearing and the other had moderate hearing loss at one or more audiometric frequency. Comparison of the DPOAE I/O function slopes between the two groups did not show any differences in their relative slope values. However this may be related to the constituents of the hearing impaired group. No information is given on their audiometric data, and although they were classified as hearing impaired their hearing may have been normal at some of the frequencies under comparison.

Dorn et al (2001) measured DPOAE I/O functions in subjects with a range of HTL. They showed an increase in I/O slope with increasing HTL, which was most marked at 4 kHz.

In a study of normal hearing children, Mulheran and Degg (1997) compared DPOAE I/O functions in normal hearing children undergoing gentamicin treatment against control subjects. They showed differences in the growth functions of the two groups, with the gentamicin group having higher slope values than the normal hearing control group.

Janssen et al (1998) measured DPOAE I/O functions in human subjects with sensorineural hearing loss and tinnitus. They correlated the slope of the DPOAE I/O function slope with HTL. If I/O function slope is related to CA function, then it is expected that the two measures would be well correlated. They showed a general relationship between HTL and I/O function slope values. Low HTL values were associated with low slope values and they increased concurrently, although there was wide variability between subjects. Kummer et al (1998) also obtained similar results correlating the I/O function slope and HTL in both normal hearing and hearing impaired subjects. There was a better relationship in the hearing impaired subjects.

Boege and Janssen (2002) performed an experiment in human subjects using a similar method to Mills (1997). DPOAE I/O functions were measured in 138 subjects with a range of hearing from normal to severe hearing impairment. They calculated DPOAE threshold by extrapolating the I/O function to give the L2 value at which DPOAE level was equal to 0 dB (this method was similar to that used by Mills (1997) to calculate changes in DPOAE I/O functions). DPOAE threshold was then correlated to the pure tone HTL at the same frequency. The relationship of the two variables had a correlation coefficient of 0.65, with a slope close to 1 dB/dB, although the between subject variability was high.

Abdala et al (2000) used DPOAE I/O functions to test the hypothesis that the CA of neonates is less developed than that of adults. They measured DPOAE I/O functions in three groups of subjects: adults, term neonates and premature neonates. Adults and term neonates were compared for differences in DPOAE level growth curve, I/O function slope and saturation threshold. They showed no difference between the I/O functions of neonates and adults. DPOAE from adults and premature neonates were also compared. The I/O functions from the premature neonates were best represented by a straight line, and showed significantly raised saturation thresholds compared to the adult functions. However there was no significant difference in I/O function slope between the groups. They concluded that neonates have less developed CA than adults. Table 2-4 summarises the results of these studies.

Longitudinal studies

An animal study is first described as it provides a useful framework for further investigations, and is similar to the approach taken by some of the cross-sectional studies of DPOAE I/O functions

described previously. Mills (1997) tested the model described earlier experimentally using the ototoxic drug furosemide in gerbils. Normal ears showed characteristic I/O functions with DPOAE level increasing in a nonlinear relationship to stimulus level. This relationship became linear only at high stimulus levels where the CA saturated. The DPOAE at high stimulus levels were predicted to be physiologically invulnerable as they were generated by passive means. Manipulation of the gain of the CA using ototoxic drugs showed I/O functions that became more linear as CA gain was reduced (Mills and Rubel, 1996). This model stipulated that the difference between pre- and post-drug I/O functions was due to a reduction in CA gain, see Figure 2-6. By quantifying this change in linearity from pre- to post-drug treatment the gain of the CA was quantified.

Interestingly, although the above general pattern was supported in the animal experimental results, the slope achieved in the notional linear condition after furosemide treatment never reached 3 dB/dB: I/O functions from cochleae with the CA totally destroyed had slopes with a maximum of 2 dB/dB. This may be because the passive generator was not truly passive at the stimulus levels used. The model was also unable to distinguish between small changes in CA function, only between moderate and severe cochlear dysfunction. The recently proposed one component model of DPOAE generation (Mills, 2002) may explain why the predictions of this model were not fulfilled. The recent study by Mom et al (2001) showed that DPOAE generated to high level stimuli in small mammals were not invulnerable may also account for these results.

Lonsbury-Martin and Martin (1990) reported a case of a patient with an idiopathic sensorineural hearing loss. DPOAE I/O functions were tracked as the patient was treated with steroids. Hearing improved at certain frequencies, and associated with this improvement was an increase in DPOAE level at these frequencies particularly at the lower intensity stimulus levels. This resulted in a shift of the DPOAE I/O function to the left of the graph and an increase in compressive nonlinearity.

Janssen et al (1998) reported the case of a 21-year-old female with tinnitus and TTS at 4 and 6 kHz from visiting a disco. They measured DPOAE I/O functions pre- and post-TTS. At frequencies between 4 and 6 kHz, there was a reduction in DPOAE level at stimulus levels of approximately 40 dB and below. This resulted in a reduction in nonlinearity of the I/O function and therefore an increase in the slope, from approximately 0.2 to 0.7 dB/dB. There was also a small change in the I/O functions at 2 and 3 kHz. These were frequencies where there was no change in hearing.

Engdahl and Kemp (1996) showed an increase in DPOAE I/O function slope in human subjects following temporary exposure to noise. This was consistent with a greater decrease in DPOAE level at the lower intensity stimulus levels. They did not relate these changes in DPOAE with changes in HTL.

2.3.2.5 DPOAE suppression tuning curves

DPOAE suppression tuning curves (STC) have been proposed as a method to estimate CA gain. This is based on their similarity to frequency and neural tuning curves, which measure the sensitivity and frequency selectivity of the cochlea.

DPOAE STC are measured by plotting DPOAE level using a particular fixed set of primary parameters. A third suppressor tone is then introduced near to the frequency of the primaries and this tone is swept in frequency and intensity, whilst measuring the resultant effect on the DPOAE level. The STC is constructed by plotting the suppressor tone level that gives a designated reduction in DPOAE level, across frequency. These curves look similar to neural tuning curves, but are fundamentally different as their properties are strongly related to the stimulus parameters used. Figure 2-7 shows an example of a DPOAE STC curve.

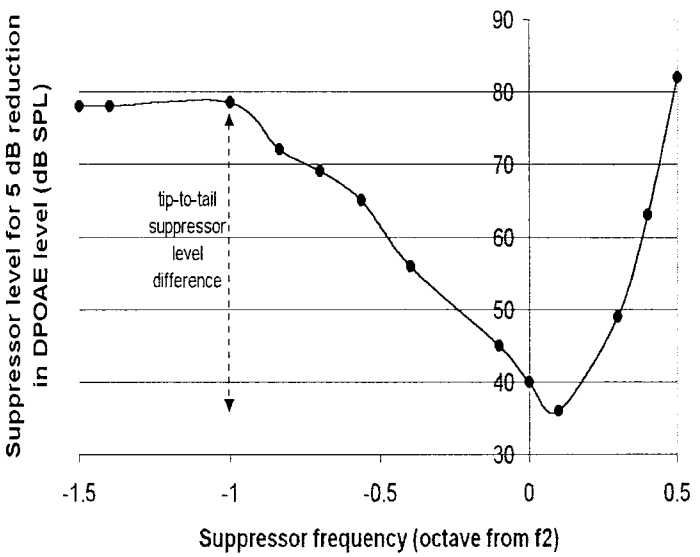


Figure 2-7: DPOAE suppression tuning curve (adapted from Pienkowski and Kunov, 2001).

Mills (1998) extended his model of I/O functions by introducing a third tone to determine the relationship between CA gain and the DPOAE STC. Mills defined important parameters of the STC that related to the CA. These were the tip-to-tail distance of the curve, which was defined as the change in suppressor level between the tip of the curve at the lowest stimulus level and the level at the shoulder point of the STC. Also he defined the tuning width (w40) which was the frequency distance between the tip of the curve and the frequency at which the DPOAE was suppressed 40 dB above this. The model predicts an approximately 1:1 relationship between STC threshold shift and the gain of the CA. Testing on gerbils showed a good agreement between the gain of the CA and the tip-to-tail distance of the STC; also between the activity width of the CA and the tuning width of the STC.

Following on from Mills (1998), Pienkowski and Kunov (2001) tested this model in human subjects. They hypothesised that there would be a significant relationship between tip-to-tail distance of the DPOAE STC and HTL. They measured STC with f_1 = 3.338 kHz and f_2 = 4.005 kHz; HTL was measured at 4 kHz. In some of these subjects they also measured DPOAE STC and HTL at 2 and 3 kHz. At 4 kHz they found a significant negative correlation between tip-to-tail distance and HTL. Linear regression of these variables at 4 kHz gave an R-square value of approximately 0.5. Correlation analysis showed a decreasing association with decreasing frequency. They concluded that although the correlation with HTL was high, STC was not a direct measure of CA gain and did not explain all the variation in hearing. However their experiment only used a small range of HTL (up to 25 dB HL) and did not include subjects with moderate or worse hearing loss. Testing across a wider range of HTL may have improved the correlation. In the correlation analysis, they also related DPOAE level with HTL at the frequency of the f_2 stimulus. They did not take into account contribution of hearing at other regions along the BM that are likely to have contributed to DPOAE activity.

Abdala et al (2001a) used a similar method to investigate the CA in human subjects. They tested the hypothesis that neonatal cochlear amplifier function is not fully mature at birth, by comparing DPOAE in neonates with those of children and adults. They measured STC at low and high primary stimulus levels hypothesising that the CA was turned on and off respectively in those conditions, to give an estimate of gain. STC from premature and term neonates, 8-12 year old children and adults were measured and compared. They showed that neonates have sharp, narrow, steep STC. Adults have STC that are broader, and children have STC similar to those of adults. The difference between the low and high stimulus STC increased with age, suggesting an increase in CA gain with age. In a subsequent study, Abdala (2001b) compared the STC between normal hearing children and adults to distinguish whether the difference in STC was due to immaturity in the children or aging in the adults. The results showed no significant difference between the DPOAE STC of adults and children, indicating that the difference to neonates is due to cochlear immaturity and not adult aging.

Gorga et al (2002) investigated the DPOAE STC method and its similarity with cochlear growth functions. They also examined the potential of this method as a measure of CA gain in human subjects. They concluded that lower input levels gave a higher measure of CA gain, consistent with the expected model. They did not correlate the gain values with HTL.

The main disadvantages of using DPOAE STC is that the exact interaction of the three tones on the BM is likely to be complex and is not yet fully understood. Although they have a similar appearance to neural tuning curves (NTC), there are differences. Firstly, NTC are generated with a single pure tone, whereas DPOAE STC are generated with three pure tones. Secondly, there is evidence that the response of STC to cochlear insult is different to that of NTC. Howard et al (2002) examined the effect of significant noise exposure on rabbit DPOAE STC. Unlike NTC that show an increase in tip

threshold and an increase in bandwidth, DPOAE STC showed a small but significant sharpening in the curve with noise exposure and an increase in tip characteristic frequency. This experiment implies that NTC and DPOAE STC are measuring different properties of the cochlea, and that they cannot be directly compared. They would appear to be unsuitable for monitoring changes in hearing.

2.3.2.6 DPOAE f2/f1 ratio curves

Another parameter originally expected to be a good estimate of cochlear tuning was DPOAE primary ratio tuning curve, measured by varying the f_2/f_1 ratio and measuring the effect on level. The DPOAE level recorded from this paradigm has a characteristic shape that resembles the BM filter pattern.

Konrad-Martin et al (1998) investigated DPOAE filter shape as a method to estimate CA gain. They examined the relationship between the filter shape at low and high intensity stimulus levels. They used furosemide to manipulate CA gain, but this had no effect on filter shape at either level, only on DPOAE level.

Engdahl and Kemp (1996) examined the effect of 105 dB SPL 2 kHz narrow band noise exposure for 10 minutes on the primary ratio tuning curve. They showed no change in the bandwidth or sharpness of the tuning curve.

Filter shape is therefore not a suitable method to probe HTL. The lack of relationship is unsurprising because the generation mechanisms for low and high f_2/f_1 ratios are known to be fundamentally different (Knight and Kemp, 2000).

2.3.2.7 Gaps in knowledge

Of the cross-sectional studies all DPOAE measures, including simple measures like level or complex measures such as the STC, have shown similar relationships with HTL. Correlation values range from 0.5 to 0.8, and the higher frequencies show the highest correlation to HTL.

Most of these experiments have been cross-sectional, and there have been relatively few longitudinal experiments. To evaluate the relationship between DPOAE and HTL fully, longitudinal experiments are the most powerful, and it is important to compare the results from cross-sectional studies with similarly designed longitudinal experiments.

The use of lower level primary tones in future studies may improve the relationship of the two variables.

2.3.3 Comparison of TEOAE and DPOAE

2.3.3.1 Human studies

Cross-sectional studies

There are few cross-sectional studies that compare TE and DPOAE responses within the same subjects. Gorga et al (1993b) compared the correlation between TE and DPOAE level in subjects with normal hearing and hearing impairment. TEOAE were evoked by click stimuli of 70 dB SPL in the nonlinear mode, and DPOAE using primary levels L1/L2 of 65/50 dB. A correlation of 0.14 was obtained at 0.5 Hz, and increased with increasing frequency. At 2 and 4 kHz, the correlation of TEOAE and DPOAE with HTL was 0.78 and 0.77 respectively.

Knight and Kemp (1999) studied the relationship between DPOAE and TEOAE level and phase. They showed that the highest correlation between DP and TEOAE level was obtained when DPOAE were evoked using a small f_2/f_1 frequency ratio, and at low stimulus levels. At small frequency ratios, DPOAE generation is dominated by reflection and therefore has more similarities to TEOAE generation.

Longitudinal studies

Although the prediction by Shera and Guinan (1999) has not been tested in humans, Berninger et al (1995) showed that quinine (which has an established effect on the cochlear amplifier) had a greater effect on TEOAE than DPOAE. However, their study used relatively high stimulus levels: DPOAE were evoked at stimulus levels of 75 dB and TEOAE at 79 dB SPL. Investigation at lower stimulus levels would have been interesting. A further study (Berninger and Gustafsson, 2000) further investigated the effect of quinine on DPOAE evoked to a range of stimulus levels. They compared the results to their earlier experiment involving TEOAE. They found no correlation between the TTS and the shifts in DPOAE level (across the range of stimulus levels used), whereas they found a high correlation with the TEOAE detection threshold. They concluded that TEOAE were more sensitive than DPOAE to changes in cochlear function.

Vinck et al (1999) compared the sensitivity of TE and DPOAE in human subjects to noise exposure. They performed two experiments: the first monitored TE and DPOAE in subjects exposed to 90 dB SPL broadband noise for 1 hour. The second experiment monitored TE and DPOAE in subjects exposed to 5 hours of disco music at L_{eq} (5 hr) of 103.5 dB SPL. TEOAE were evoked by click levels of 70 dB SPL in the nonlinear mode, and DPOAE were evoked by L1/L2 primary levels of 65/55 dB SPL at frequencies ranging from 0.6 to 5.5 kHz. In the first experiment, there was no significant change in HTL, and the group mean hearing threshold shift was less than 5 dB. However TEOAE showed a significant reduction in total response level (mean 2.5 dB). There was a reduction in

TEOAE SNR at 4 kHz (mean 4 dB), but no significant changes at other frequency bands. DPOAE were significantly reduced in level between 3.9 kHz and 5.5 kHz (range 7-9 dB).

In the second experiment, there was a significant group mean hearing threshold shift of 20 dB between 4 to 6 kHz. There was a significant reduction in TEOAE total response level (approx 6 dB mean), and a reduction in SNR at each frequency band with the largest change at 4 kHz. DPOAE were significantly reduced between 3 and 5.5 kHz, and showed a mean reduction of 9 dB. Vinck et al (1999) concluded that OAE are more sensitive to changes in hearing than pure tone audiometry. They also concluded that both types of OAE are frequency specific to the audiometric changes at 4 kHz. Their experiment did not compare the changes in individual subjects, nor directly compare the changes in TE and DPOAE within subjects. Details of the audiometry methodology are not described in detail, so the step size used is assumed to be 5 dB. Although they conclude that OAE are sensitive to pre-audiometric changes, audiometry using smaller step sizes may have shown subtle changes in the audiogram, particularly in experiment one.

Marshall et al (2002) examined changes in HTL, TE and DPOAE in 339 sailors before and after a six-month deployment on an aircraft carrier where they were exposed to significant noise levels. TEOAE were evoked by a click stimulus level of 74 dB SPL. DPOAE were evoked by four different stimulus levels (L1/L2 65/45, 61/55, 59/50 and 57/45 dB) at f_2 frequencies between 1.8 to 4 kHz. There was no significant change in the group mean HTL, although 18 ears showed permanent threshold shifts. There were significant changes in TEOAE level with the largest change at 4 kHz. There were also significant changes in DPOAE level. Marshall et al (2002) found no agreement between the change in HTL and the change in either of the OAE types. They also classified the ears according to whether there was a change in hearing and/or a change in OAE level. Of 150 ears, 29 showed no change in hearing but a significant change in TEOAE, whereas 40 showed no change in hearing but a significant change in DPOAE. Interestingly, seven ears showed a significant change in hearing but no significant change in TEOAE. Six ears showed a significant change in hearing but no significant change in DPOAE. They concluded that a change in TE or DPOAE was not a good predictor of a change in hearing. They do not give details of the audiometry step sizes used, which are therefore assumed to be 5 dB and may not have been sensitive enough to detect small changes in hearing. Comparison of the changes in TE and DPOAE showed a significant correlation (0.3-0.6). They do not provide individual subject analysis, particularly of the susceptible subjects.

2.3.3.2 Animal studies

There are several studies comparing the effect of changes/differences in cochlear function on OAE type in animals. For this reason relevant animal studies are briefly reviewed here, although it is acknowledged that OAE generation in animals is slightly different to that of humans.

Kakigi et al (1998) compared the effect of aminoglycoside treatment on TE and DPOAE recorded from chinchilla ears. Auditory brainstem response (ABR) threshold was measured at audiometric frequencies using tone pip stimuli and OHC loss along the cochlea was also measured. They showed no significant relationship between the group change in ABR threshold and either OAE response. The authors did not analyse individual animal changes in ABR and OAE. In two chinchillas that had hearing loss at one frequency only, there was a reduction in DPOAE but not TEOAE level. This may be explained by the fact that a relatively high stimulus level of 80 dB (insensitive to cochlear dysfunction) was used to evoke TEOAE whilst a relatively low stimulus level of 50 dB (sensitive to dysfunction) was used to evoke DPOAE. They found no significant correlation between DP and TEOAE signal-to-noise ratio recorded from the chinchilla, which may also be explained by the stimulus levels used.

Sockalingam et al (2000) compared TE and DPOAE in three species of guinea pigs treated with cisplatin. ABR threshold was also measured. There was a significant group correlation between DPOAE level and ABR threshold in two of the species. Significant correlation coefficient values ranged from -0.79 to 0.96, although they were only significant at $P<0.05$. There were also significant correlations in two species between TEOAE (2-4 kHz) and DPOAE (2, 3, 4 and 6 kHz), although similarly correlations were only significant at $P<0.05$. The reasons that significant correlations were recorded in this experiment may be that TEOAE were evoked by low intensity level stimulus of 65 dB SPL. DPOAE were evoked by L1/L2 primary levels of 50/40 dB SPL.

Fraenkel et al (2001) also compared changes in TEOAE, DPOAE and ABR threshold in rats following noise exposure. They were exposed to 113 dB SPL for 12 hours per day and were monitored for up 21 days. TEOAE were evoked by click levels of 65, 60 and 55 dB SPL; DPOAE by L1 of 60, 50 and 40 dB, with L2 10 dB lower in each case. They showed a significant increase in ABR threshold over the duration of the experiment. There was no dependence of TE or DPOAE on noise exposure, which reached the maximum reduction in level after 3 days exposure. The authors did not correlate the change in OAE with the change in ABR threshold. Neither do they report the relationship between the change in TE and DPOAE, either in individual animals or over the whole group.

2.3.3.3 Gaps in knowledge

It is clear from the evidence reviewed here that the relative effects of increased HTL on TEOAE and DPOAE in humans cannot be predicted with certainty from existing models. Limited experimental evidence is available from studies in humans. There is a need for further evidence from humans, and parallel studies of DP and TEOAE in the same subject. Animal studies are also limited and do not show a consistent relationship between changes in hearing and changes in OAE.

The studies discussed previously showed marked differences in results, and when comparing DP and TEOAE it is likely that the variation in stimulus parameters contributes to the variation in recorded results. In longitudinal studies, it is important to examine both the group and individual changes. It is also important to make detailed comparison of concurrent changes in TE and DPOAE. Stimulus parameters are crucial, and so it is important to examine changes in OAE using a wide range of stimulus levels.

3 RATIONALE

This chapter describes the framework of the thesis. The hypotheses are given, and a conceptual diagram relating otoacoustic emissions (OAE) and hearing threshold level (HTL) is shown. The chosen OAE measures are discussed and the aims and objectives of the thesis are described in detail.

3.1 FRAMEWORK

A framework is described here relating OAE and HTL. According to Talmadge et al (1998) "OAE are believed to be generated by the same processes responsible for the remarkable sensitivity of the human ear". These processes are the cochlear amplification processes. This commonality of the cochlear amplifier (CA) for both hearing sensitivity and OAE generation suggests a close relationship between OAE and HTL.

The main assertion on which the thesis is based is that OAE and HTL (up to mild sensorineural hearing loss) are tightly coupled through the outer hair cells (OHC) and the CA. Mild sensorineural hearing loss, particularly as a result of noise damage is likely to be predominantly OHC loss (Wright et al, 1987; Schuknecht and Gacek, 1993; Borg et al, 1995) and OAE generation requires cochlear amplification (Talmadge et al, 1998; 2000), and is thought to reflect OHC loss (Avan et al, 1993). Based on this evidence, a high correlation between OAE and HTL is expected. Alternatively, other factors may also be involved in both hearing sensitivity and OAE generation and these may make a significant contribution to the relationship between OAE and HTL. These factors may include cochlear nonlinearities and inhomogeneities for OAE generation, and IHC and other auditory processes for HTL. If these other factors are involved, a weak to moderate correlation between OAE and HTL is expected.

Previous experimental studies examining the relationship between OAE and HTL have shown only a moderate correlation between the two (e.g. Gorga et al, 1993a, b). It is therefore proposed that the moderate correlations shown in previous studies are the result of a poor choice of OAE and HTL measures. Many of the experimental studies have limitations, notably the use of high-level stimuli. The use of improved OAE measures is hypothesised to increase the correlation between OAE and HTL over previous studies.

Secondly, it is proposed that the moderate correlations recorded in the previous studies are also the result of inter-subject and inter-ear variability in OAE and HTL measures, affecting them in different ways. Most previous studies have been cross-sectional, which does not control for individual subject factors such as differences in external and middle ear conductivity. It is hypothesised that a longitudinal study investigating changes in OAE and HTL will show higher correlations than cross-sectional differences.

These hypotheses provide a testable framework for the thesis.

3.1.1 Assumption

One of the assumptions made for the purposes of this thesis is that for mild degrees of sensorineural hearing impairment, the site of the impairment is mainly the OHC with little involvement of the IHC or other cochlear or neural auditory processes. This assumption is encompassed in a diagram of the proposed relationship between OAE generation and HTL (up to mild sensorineural hearing impairment), mediated through the CA (Figure 3-1). The cochlear amplifier as used in this thesis refers to the overall functioning of the amplification process, and does not specify a particular mechanism. The efferent auditory system can moderate the output of the CA, and so efferent activity is included within the overall definition of the CA.

Figure 3-1 represents diagrammatically this assumption based on the CA having a key role in both OAE and HTL, and changes in cochlear amplification are hypothesised to affect OAE and HTL equally. Alternatives to this assumption are acknowledged in the diagram, and other factors that may influence the relationship as well as the CA include cochlear nonlinearities and inhomogeneities for OAE generation, and inner hair cells and neural auditory processing for HTL. These are also shown.

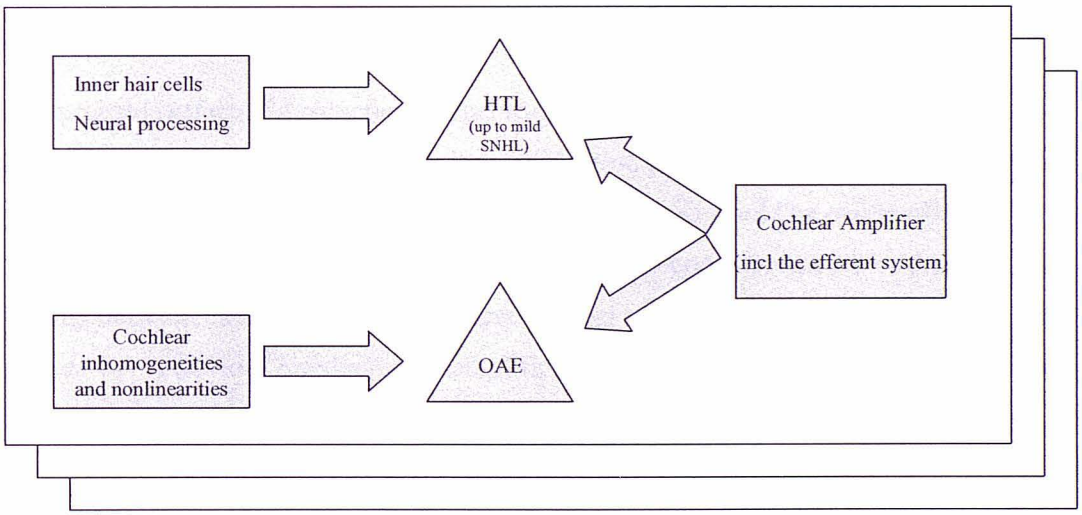


Figure 3-1: Conceptual diagram of the relationship between HTL and OAE, in which the cochlear amplifier has a key role in both OAE and HTL, and changes in cochlear amplification are hypothesised to affect OAE and HTL equally (shown on the right hand side of the diagram). Other factors that may influence the relationship as well as the cochlear amplifier are shown on the left hand side of the diagram.

3.1.2 TEOAE and cochlear amplification

The effect of a change in the CA on TEOAE generation is unclear. A conceptual diagram representing the relationship between TEOAE and the CA is shown in Figure 3-2. It is a simplistic, schematic diagram that proposes transient evoked (TE) OAE generation along the basilar membrane (BM) at low and high stimulus levels, and for normal and reduced cochlear amplification levels. This is a conceptual representation only and not drawn to scale, it is also deliberately simplistic. TEOAE generation is described for a single frequency component, and this process is expected to be duplicated for other wavelets across the frequencies.

At low stimulus levels, TEOAE production in ears with normal CA function is assumed to be dominated by reflection of the tall and broad travelling wave (TW) from the irregular perturbations of the CA. In addition, intermodulation distortion may generate apical and basal TW. The basal TW adds to the measured TEOAE; the apical TW travels to its characteristic site, where it is reflected and contributes to the TEOAE. At low stimulus levels the contribution of distortion to the overall TEAOE is likely to be small. However at high stimulus levels, the contribution to the TEOAE from intermodulation distortion is proposed to be greater than at low levels (Talmadge et al, 2000). The reflection contribution is likely to be smaller as the tall and broad requirement for coherent reflection filtering is not fulfilled to the same degree at high stimulus levels.

The probable effect of a reduction in cochlear amplification on TEOAE generation is also described. At low stimulus levels, the reduction in amplification implies the TW produced is not so finely tuned and does not have the same tall and broad characteristic as would be expected with normal CA function. This is likely to lead to a reduction in the reflection component of the TEOAE. Intermodulation distortion may still generate apical and basal TW although reduced compared to the normal CA condition. A reduction in amplification at other places along the BM will reduce the reflection of these components. At high stimulus levels, the reflection contribution will be reduced compared to the normal CA function condition. The distortion contribution may also be slightly reduced, and reflection of these distortion components will also reduce.

The effect of hearing level on TEOAE level across the frequency spectrum has been investigated. Avan et al (1997) showed that high frequency hearing loss outside the frequency range of the TEOAE spectrum contributed to variability in the TEOAE level at lower frequencies. They proposed this was due to distributed source generation of TEOAE, with CA function at other frequencies contributing to the overall TEOAE. However, this was only investigated at a relatively high intensity stimulus level. This thesis aims to extend that work and it is hypothesised that the influence of the CA from distributed sources along the BM on TEOAE generation depends on the contribution of reflection and distortion components to the generation mechanism (Avan et al, 1997; Yates and Withnell, 1999). The relative contribution of reflection and distortion depends on stimulus level. Variability in the

TEOAE spectrum and hearing threshold level (HTL) is investigated across a range of stimulus levels. This is best tested through examining changes in CA function and TEOAE spectrum.

The framework relating TEOAE level to CA function across the frequency range is based on the theory that TEOAE are generated from both reflection and distortion sources. It is hypothesised that at lower stimulus levels, TEOAE are mainly generated from single channel reflection sources, and that there is little contribution of distributed sources to the variation in the spectrum. High frequency hearing loss will therefore make little contribution to the variability in the TEOAE spectrum. However at high stimulus levels, when distortion may be important to TEOAE generation, high frequency hearing may have an effect on TEOAE generation. Figure 3-3 represents this diagrammatically.

3.1.3 DPOAE and cochlear amplification

A schematic diagram is proposed for DPOAE generation in Figure 3-4. This is a conceptual representation only and not drawn to scale, it is also deliberately simplistic. Intermodulation distortion of the primary stimulus frequencies results in the generation of distortion product OAE. This generates apical and basal TW at intermodulation frequencies. At low stimulus levels, DPOAE production in ears with normal CA function is a combination of distortion and reflection of the intermodulation distortion product at its characteristic frequency. The exact contribution of the two mechanisms is not known. At high stimulus levels, DPOAE is likely to be dominated by the distortion component. In ears with reduced CA function, the effect on the relative contributions to DPOAE can only be surmised. According to Shera and Guinan (1999), a reduction in CA gain will affect the reflection more than the distortion mechanisms. The relative effect on reflection sources is likely to be greater at low stimulus levels.

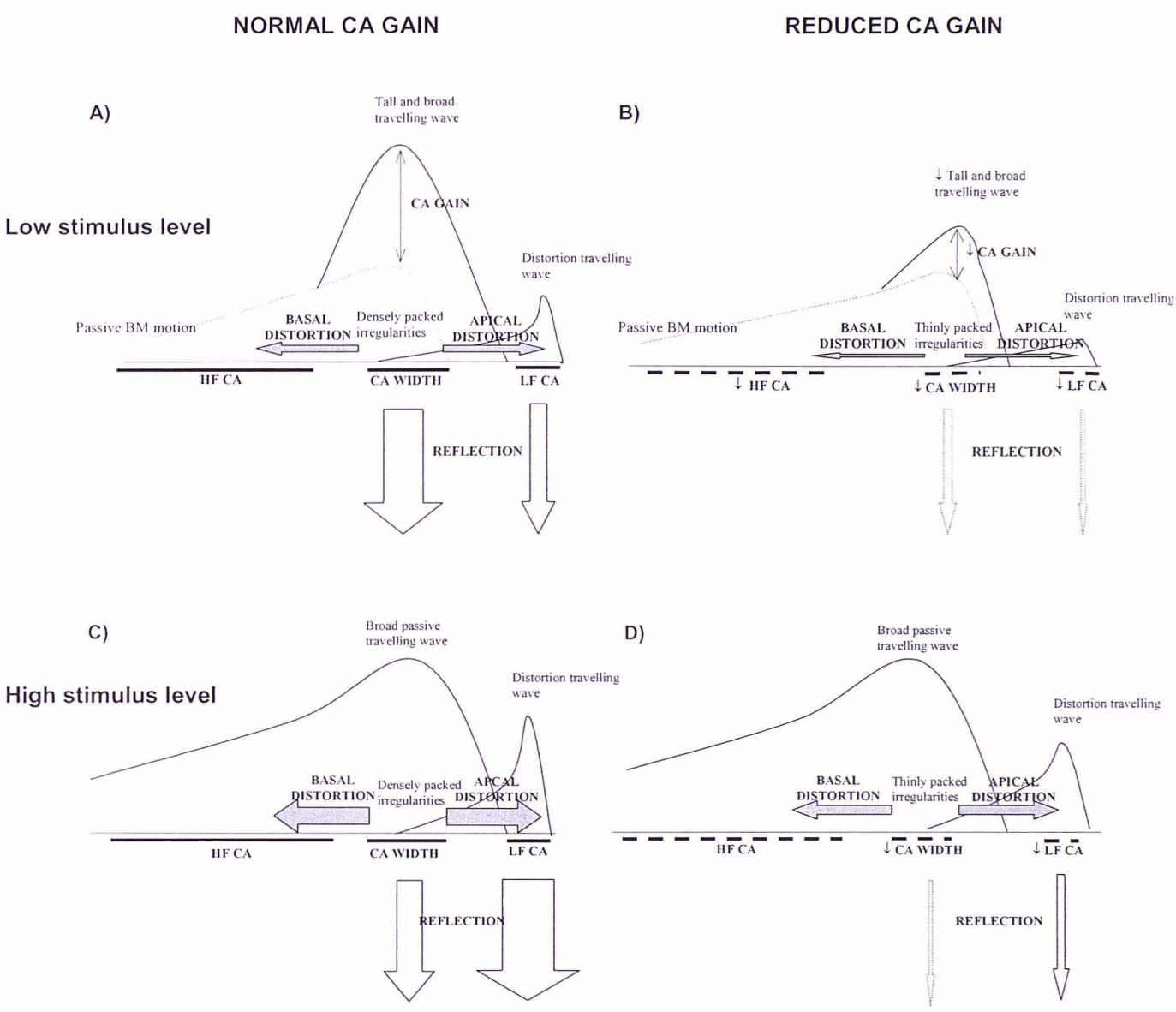


Figure 3-2: Conceptual diagram showing TEOAE production at low and high stimulus levels at normal CA gain (i.e. normal hearing) and reduced CA gain (i.e. mild sensory hearing impairment). A) Low TEOAE stimulus level, normal CA gain. It is hypothesised that the reflection mechanism dominates TEOAE generation. B) Low TEOAE stimulus level, reduced CA gain. It is hypothesised that the reflection mechanism is more sensitive to changes in CA gain than the distortion mechanism, so there is a large difference when compared to the normal CA gain case at low stimulus levels. C) High TEOAE stimulus level, normal CA gain. It is hypothesised that the distortion mechanism dominates TEOAE generation D) High TEOAE stimulus level, reduced CA gain. It is hypothesised that the distortion mechanism is less sensitive to changes in CA gain than the reflection mechanism, so there is a small difference when compared to the normal CA gain case at low stimulus levels. Key to abbreviations – LF: low frequency, HF: high frequency, CA: cochlear amplifier, BM: basilar membrane.

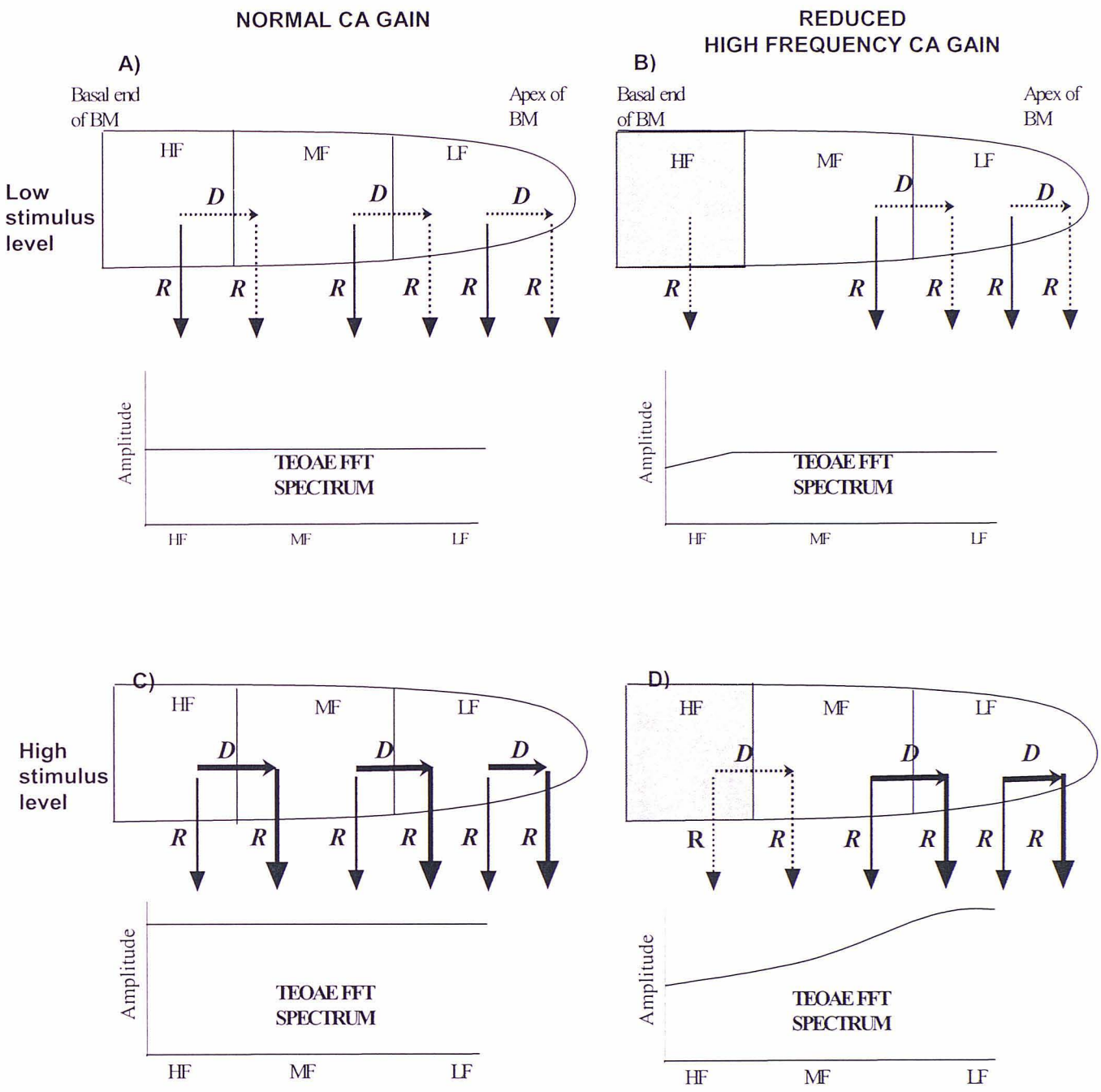


Figure 3-3 Diagrammatic representation of TEOAE generation. A) TEOAE generated by a low level intensity click from a normal hearing cochlea. The main generation source is reflection. B) TEOAE generated by a low-intensity level click from a cochlea with a high frequency hearing loss. There is a reduction in reflection from the area with a hearing loss. The distortion contribution to TEOAE generation is minimal at low-levels, so it is likely to have less impact on the lower frequencies of the spectrum. C) TEOAE generated by a high-intensity level click from a normal hearing cochlea. As well as generation from reflection at frequency specific areas, there is also contribution from apically generated distortion sources. D) TEOAE generated by a high-intensity level click from a cochlea with a high frequency hearing loss (shaded area). There is a reduction in reflection from the area with a hearing loss, and there is also a reduction in distortion generated from the high frequency area. Key to abbreviations – R: reflection D: distortion, HF: high frequency, MF: mid frequency, LF:low frequency.

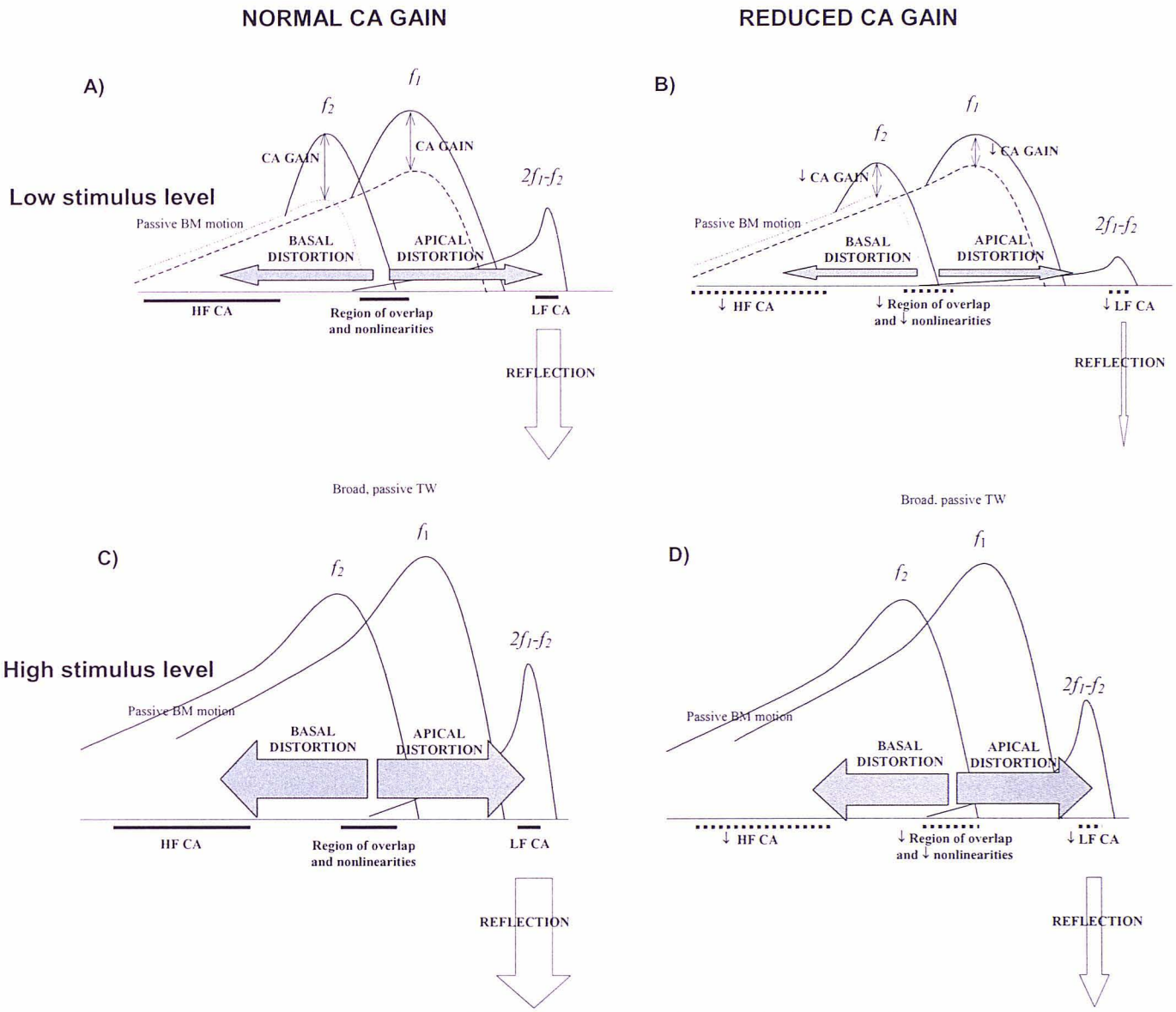


Figure 3-4: Conceptual diagram showing DPOAE production at low and high stimulus levels at normal CA gain (i.e. normal hearing) and reduced CA gain (i.e. mild sensory hearing impairment). A) Low DPOAE stimulus level, normal CA gain. It is hypothesised that the reflection component is proportionally higher compared to the high stimulus level. B) Low DPOAE stimulus level, reduced CA gain. It is hypothesised that both the reflection and distortion components are sensitive to the reduction in CA gain and there is a large difference when compared to the normal CA gain.case. C) High DPOAE stimulus level, normal CA gain. The reflection and distortion components both contribute markedly to the DPOAE D) High DPOAE stimulus level, reduced CA gain. At high stimulus levels it is hypothesised that mainly the reflection component is sensitive to changes in CA gain, so there is a small difference when compared to the normal CA gain case at high stimulus levels.. Key to abbreviations – LF: low frequency, HF: high frequency, CA: cochlear amplifier, BM: basilar membrane.

3.2 OAE MEASURES

In the following section, the OAE measures are described which will be related to HTL. For simplicity, the OAE models described do not take the contribution from distributed frequency sources into account. The importance of distributed frequencies will ascertained by the frequency relationship of the different frequency OAE measures with HTL.

3.2.1 OAE I/O functions

OAE I/O functions will be used to investigate the relationship between OAE and HTL as the relationship between OAE and stimulus level is likely to give information on CA function. The CA is level dependent and makes a significant contribution at low to moderate stimulus levels. Therefore it is expected that the correlation between OAE with HTL will be higher when OAE are measured at lower stimulus levels.

A framework based on Mills' model (1997) of DPOAE I/O functions in gerbils is used for investigation in human subjects. There is recent evidence showing deficiencies in Mills' 1997 model (Mills, 2002). However this mainly refers to the origin of the notch in the functions at mid to high stimulus intensity levels. Animal DPOAE are now thought to arise from a one-source generation mechanism rather than the two source active and passive generation mechanism. Mills (2002) attributes the notch in DPOAE I/O functions to the shape of the travelling wave at high intensity levels and not an interaction between active and passive DPOAE. This new interpretation of the model does not significantly alter the overall concept at the lower stimulus levels of the I/O function where the CA is maximally functional.

A similar approach to Mills (1997) has not been taken in human subjects, although there are cross-sectional data relating DPOAE I/O functions and HTL in human subjects that support this approach (Dorn et al, 2001). TEOAE I/O functions have not been investigated in this way.

The framework is intentionally simple and will be used for estimating changes/differences in OAE in human subjects. The low-level, nonlinear portion of the I/O function is likely to be where the CA is non-saturating and predicted to be most sensitive to cochlear dysfunction. Where the I/O function plateaus is likely to be where the CA is saturated, and thus less sensitive to cochlear dysfunction.

As well as I/O function growth and saturation, OAE level and detection threshold can also be evaluated through this model. Using I/O functions ensures that similar measures can be made using both DP and TEOAE, allowing comparison between the two measures. In the present work, new technology DPOAE equipment is used, and TEOAE are recorded using maximum length sequences. This allows measurement of OAE to low stimulus levels.

3.2.1.1 DPOAE

The framework relating DPOAE level and HTL will be based, with modifications, on Mills' model (1997) of DPOAE I/O functions in gerbils, see Figure 2-6. DPOAE measurements will be taken according to the framework and correlated with HTL.

DPOAE I/O functions are similar to the I/O functions relating basilar membrane (BM) vibration to stimulus level. In normal hearing subjects, BM vibration at low-intensity stimulus levels is predominantly a result of active amplification of the TW, and has a compressive nonlinear relationship with stimulus level as the amplification process saturates. At high-intensity levels, BM movement derives mainly from passive vibration and has a linear relationship with the stimulus. There is evidence that at very low intensity level stimulus levels, BM vibration is also linear (Robles and Ruggero, 2001). This results in an I/O function that is compressively nonlinear at the low- to mid-intensity levels. In hearing impaired subjects, there is a reduction in vibration at low intensity levels resulting in an increase in linearity of the I/O function.

For DPOAE I/O functions, the morphology is similar. In normal hearing subjects there is an increase in DPOAE level with increasing stimulus level. DPOAE growth with stimulus level is compressive over the mid-intensity levels and saturates at high levels. In hearing impaired subjects, overall DPOAE level is reduced, there is little or no compression and less likelihood of saturation (Dorn et al, 2001). The slope of the DPOAE I/O function in normally hearing subjects is often less than 1 dB/dB and in subjects with hearing impairment, the slope can reach a maximum of 1.5 to 2 dB/dB.

These differences in DPOAE I/O functions between normal hearing and hearing impaired subjects are thought to arise from the different sensitivities of DPOAE evoked by low and high intensity level stimulus levels. DPOAE evoked by high intensity tones are considered to be evoked mainly from passive mechanisms. At these stimulus levels CA gain is effectively zero. DPOAE evoked by low intensity tones arise mainly from active mechanisms where CA gain is maximal. Therefore the relationship with HTL is likely to be more highly correlated at lower stimulus levels.

DPOAE growth at high stimulus levels is hypothesised to have a growth rate of 3 dB/dB (see **Appendix 1** for demonstration). At very low intensity stimulus levels DPOAE growth is also thought to have a growth rate of 3 dB/dB, where BM vibration is linear with stimulus. As stimulus level increases, DPOAE level shows a reduced growth rate with increasing stimulus level as the CA saturates at moderate to high stimulus levels.

A reduction in effective CA gain is likely to reduce the amplification from the CA place, generating a reduction in the overall DPOAE level particularly at the lower intensity stimulus level. However this reduction in CA gain is likely to have little effect on DPOAE generation at high intensity levels.

In this model for humans, the maximal contribution to DPOAE generation is assumed to be at the f_2 region. The model does not acknowledge the contributions of the CA from the reflection site at $2f_1-f_2$ (unlike humans, gerbils do not have a significant contribution from the f_{dp} region). It is acknowledged that in human subjects, comparing two I/O functions may confuse the importance of the contributions from the CA at the distortion site and the CA at the reflection site.

A method for estimating the change in OAE in human subjects is shown in Figure 3-5. This can be calculated in two ways: firstly as the change/difference in stimulus level required to elicit DPOAE at a fixed level. This is termed here *DPOAE stimulus level*. Secondly as the change/difference in DPOAE level at a fixed stimulus level. This is termed here *DPOAE level*.

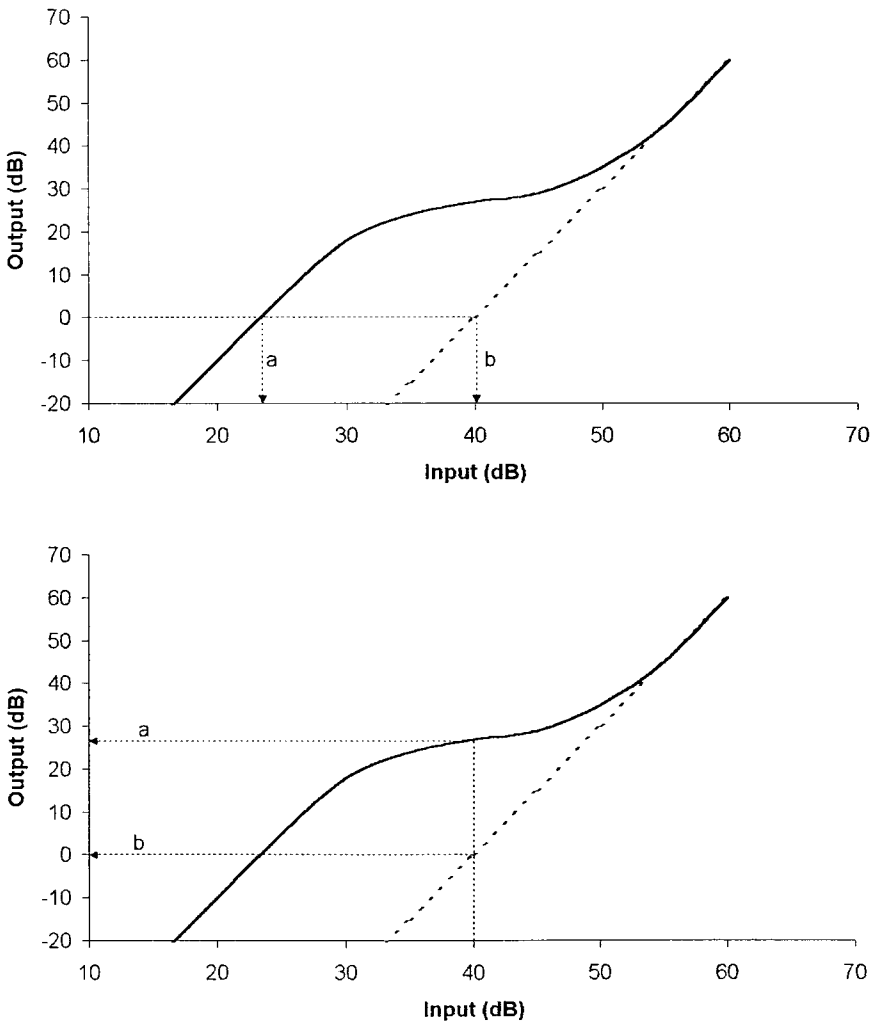


Figure 3-5 Method for estimating the change in DPOAE from I/O functions in human subjects (adapted from Mills, 1997). Solid curve shows the theoretical pre-exposure I/O function, dashed curve shows the theoretical post-exposure I/O function. The change in DPOAE can be calculated as shown in A) as the difference in the input level (a – b) required to generate a specified output level (DPOAE stimulus level method) or as B) as the difference in output (a – b) at a specified input level (DPOAE level method).

The CA place is defined as the position of the primary tone f_2. It is recognised that there are contributions to DPOAE generation from both distortion and reflection mechanisms at different sites along the cochlea (e.g. the $2f_1$–f_2 site) but these are not specified in the framework. It is acknowledged that at high stimulus levels associated with little/no active amplification, the maximum peak of the TW shifts position towards the apex. This effect is also ignored in the framework. For investigating changes/differences in DPOAE, this simple model is thought to be sufficient, at least on a first approximation and it is not required to distinguish between distortion and reflection generation mechanisms to DPOAE generation.

3.2.1.2 TEOAE

The essential characteristics of Mills' model of DPOAE I/O functions are adapted here for TEOAE. For this model, TEOAE are assumed to be generated from single channel sources. It is acknowledged that there is evidence that intermodulation distortion may also be involved in TEOAE generation (Yates and Withnell, 1999), particularly at high stimulus levels. For simplicity a single place mechanism is assumed. The place of the CA is assumed to be at the frequency of the TEOAE under investigation. TEOAE I/O functions will be represented by the compressive growth equation (as Kapadia and Lutman, 2001).

In the TEOAE case, the predicted slope value of the I/O function without cochlear amplification will not be 3 dB/dB. Following the notion of a linear reflection process proposed by Shera and Guinan (1999), the linear TEOAE case will be represented by a slope of 1 dB/dB. In the nonlinear case where there is active amplification, the slope will be reduced in accordance with the broadening of the TW envelope and reduction of gain as stimulus level is increased. Slope values below 1 dB/dB are consistent with the broad body of literature in TEOAE I/O functions in ears with normal hearing.

TEOAE evoked with low intensity stimuli are more sensitive to differences in HTL than those evoked with high stimuli (Marshall and Heller, 1996). It is therefore assumed that cochlear amplification at the higher stimulus levels is lower than at lower stimulus levels. Figure 3-6 describes theoretical I/O functions, based on linear reflection with active amplification greatest at low stimulus levels. Linear reflection is modelled as a growth function with a slope of 1 dB/dB. CA gain decreases with increasing stimulus level, therefore the effect of cochlear amplification is modelled as a slope growth function with a slope less than 1 dB/dB. An increase in CA gain is hypothesised to result in an decrease in I/O function slope. All input and output levels used are nominal.

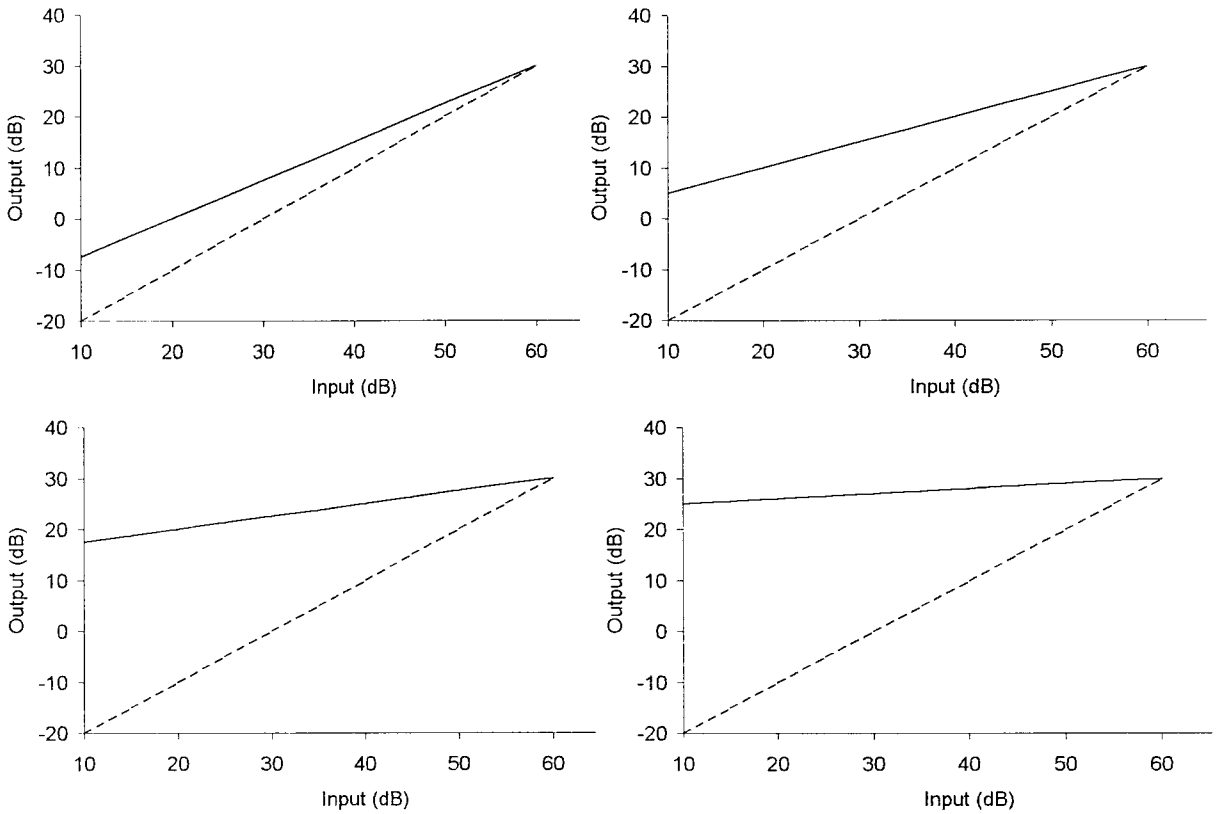


Figure 3-6: Hypothetical differences in TEOAE I/O functions with different contributions of cochlear amplification. The I/O function with minimal cochlear amplification has a slope (m) of 1 dB/dB, as shown by the dashed line labelled "linear reflection"; the active I/O function has with different contributions of cochlear amplification have different slope values "m". Greater cochlear amplification is shown by lower slope values of the solid line labelled "active + linear" A) m=0.75. B) m=0.5. C) m=0.25. D) m=0.1.

Figure 3-7 shows the methods for estimating changes in TEOAE. The change in TEOAE is defined as the difference in the stimulus level required to generate a specified TEOAE level or the difference in TEOAE level at a specified stimulus level. No effect of frequency is given.

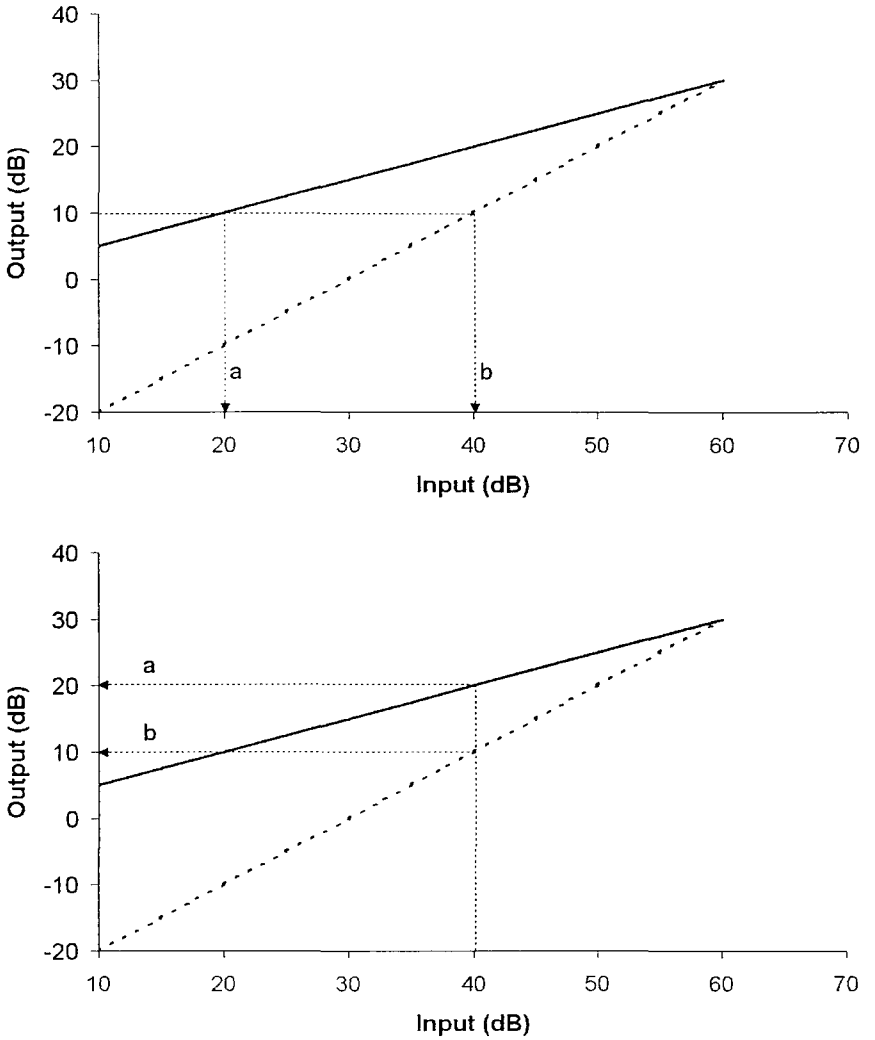


Figure 3-7 Estimating changes in TEOAE from I/O functions. Solid curve shows the theoretical pre-exposure I/O function, dashed curve shows the theoretical post-exposure I/O function. The change in TEOAE can be calculated as shown in A) as the difference in the input level (a – b) required to generate a specified output level (TEOAE stimulus level method) or as B) as the difference in output (a – b) at a specified input level (TEOAE level method).

3.2.2 TEOAE rate suppression

The phenomenon of TEOAE rate suppression is thought by some to arise from the same cochlear nonlinearities that generate the I/O function (e.g. Hine and Thornton, 1997). Rate suppression may therefore be a suitable model for relating OAE to HTL. Studies such as those by Hine and Thornton (1997) and Rasmussen et al (1998) have examined the relationship between rate suppression and the OAE I/O function in cross-sectional studies of human subjects but this has not been fully explored. The relationship between rate suppression and HTL has also not been fully examined. TEOAE rate suppression is examined for estimating differences/changes in HTL.

MLS TEOAE rate suppression is hypothesised to be related to cochlear nonlinearity. The framework relating rate suppression and HTL is based on the model proposed by Kapadia and Lutman (2001).

In normal hearing subjects, there is an increase in TEOAE level with decreasing click rate in the same way as TEOAE I/O functions, and is hypothesised to be related to the nonlinearities of the cochlear amplifier. Kapadia and Lutman (2001) predict a relationship between the TEOAE I/O function (TEOAE level versus stimulus intensity) and the rate suppression function (TEOAE level versus rate). The model predicts a decrease in rate suppression with increasing linearity of the input-output function, see Figure 2-5.

In the same way that TEOAE I/O functions are proposed to relate to HTL, rate suppression functions may also be a suitable method for examining HTL. The methodology for applying this in practice is shown in Figure 3-8. This is a conceptual diagram showing methodology and does not necessarily relate to physiological data. It is predicted that with a linear I/O function, with slope (m) equal to 1, rate suppression will be 0. This will be examined in human subjects, and the hypothesis tested that rate suppression is related to changes/differences in HTL. The effect of frequency is not modelled here.

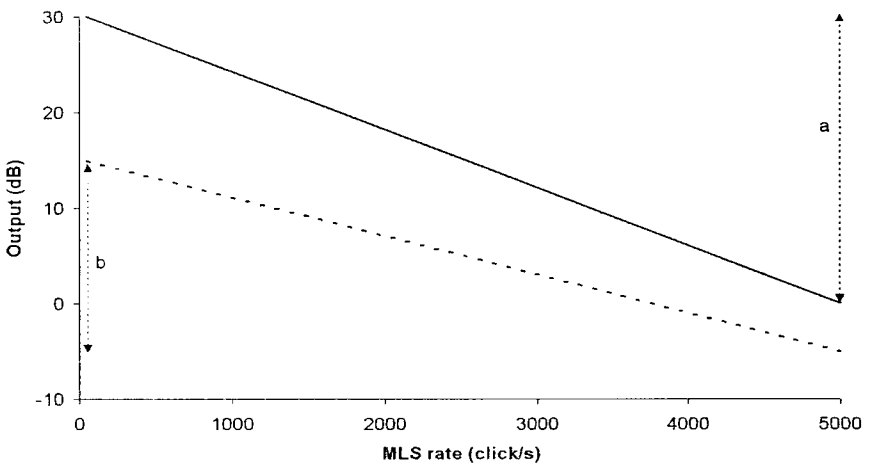


Figure 3-8: A conceptual diagram for estimating changes in MLS TEOAE rate suppression. Solid curve shows the theoretical pre-exposure MLS TEOAE, where there is a large difference in TEOAE level recorded at 50 compared to 5000 clicks/s. The dashed curve shows the theoretical post-exposure MLS TEOAE, in which there is a smaller difference in TEOAE level recorded at 50 compared to 5000 clicks/s. The change in MLS TEOAE rate suppression can be calculated as the difference in the rate suppression (a – b). This is a hypothetical relationship for illustrating methodology.

3.2.3 Comparison of DPOAE and TEOAE

The generation mechanisms of DP and TEOAE are still not fully understood, particularly with regard to their relative differences and similarities. Examination of the differential changes in DP and TEOAE with a change in hearing provides evidence as to which OAE type is most closely linked to auditory sensitivity.

3.3 AIMS AND OBJECTIVES

The main assumption of this thesis is that over the range of hearing thresholds studied (normal to mild sensorineural hearing loss), any differences in hearing are due primarily to OHC dysfunction. As cochlear amplification by the OHC is one of the main factors important for OAE generation, it is hypothesised that OAE and HTL will be highly correlated. This approach has been taken by, for example Dorn et al (2001), Pienkowski and Kunov (2001) and Boege and Janssen (2002). The alternative to this assumption is that other factors that have a differential effect on hearing sensitivity and OAE generation are important in the relationship. If this is the case, a high correlation between OAE and HTL is not expected.

OAE measures that are closely related to CA function (e.g. OAE evoked to lower level stimuli) are expected to show a higher correlation with HTL than OAE measures that are not so closely related to the CA (e.g. OAE evoked to higher level stimuli). The control of individual subject differences (e.g. middle ear factors) by longitudinal experimental design is also expected to increase the correlation of OAE and HTL over a cross-sectional design.

OAE measures and their relationship with hearing threshold will be fully investigated in human subjects. Previous studies that have investigated the relationship between OAE and HTL have not fully addressed all the issues highlighted in the previous chapters:

- CA function is level dependent and the CA is more active at low intensity levels. Most previous studies have used high-level stimulus levels to evoke OAE with little investigation of lower-level stimuli.
- Many studies have been limited by the noise floor of the equipment or test room, and have not been able to record OAE at low levels where the CA is most active.
- There has been little or no consideration of cochlear fine structure in experimental design. Cochlear fine structure can affect OAE level independent of changes/differences in HTL.
- The relationship between OAE and HTL has been investigated in many studies. These have mainly focused on OAE level and have shown moderately significant relationships between OAE and HTL. More recently, other studies have used more complex OAE measures (e.g., suppression tuning curves) but these have not increased the correlation between OAE and HTL.
- Most of the studies reported in the literature have been cross-sectional. From these experiments it is not possible to distinguish the within-subject variability from the between-subject variability. There are few reported longitudinal studies, and most of those reported in the literature used small sample sizes and monitored changes over a small number of time points.

- There is a need to study TE and DPOAE together. Most studies have investigated either TEOAE or DPOAE, but have not addressed the relationship between the two OAE types. Differences and changes in HTL should be represented in both TE and DPOAE.

Based on the literature review, several models of OAE have been chosen for further exploration in human subjects and their relationship with HTL. Two general approaches will be used: examining differences *between* subjects with differing HTL and inducing a hearing threshold shift *within* subjects. The relationship of OAE with HTL will be examined. The measures proposed in this thesis are expected to show a higher correlation with HTL than previous measures reported in the literature as they are more closely related to cochlear amplification. Longitudinal changes in OAE and HTL are expected to show a higher correlation than cross-sectional differences in OAE and HTL.

3.3.1 Aims

The aims of the thesis therefore are to:

1. Explore in human subjects the relationship between the OAE and HTL by examination of:
    a. Differences in OAE between subjects with differing HTL according to identified models.
    b. Changes in OAE within subjects with changing HTL according to identified models.
2. Examine whether differences in OAE between individuals are similar to changes within individuals.
3. Evaluate the use of OAE for monitoring the effects of ototoxic substances on hearing.

3.3.2 Objectives

The objectives of the thesis are to:

1. Characterise OAE in normal hearing subjects, across a wide range of stimulus levels, using DP and TEOAE.
2. Characterise the differences in OAE between subjects with differing HTL, across a range of stimulus levels and rates, taking into account cochlear fine structure.
3. Characterise the changes in OAE within subjects developing a temporary HTL shift, across a range of stimulus levels and rates, taking into account cochlear fine structure.
4. Compare the differences in OAE with differing HTL with the changes in OAE with changing HTL.
5. Compare differences in DP and TEOAE in subjects with differing HTL.
6. Compare changes in DP and TEOAE in subjects with changing HTL.
7. Critically evaluate the existing models of OAE in the light of the results.

4 METHODOLOGY

4.1 INTRODUCTION

This chapter describes the general methodology and equipment used in the experiments supporting this thesis. Detailed methodologies relating to individual experiments are described in the appropriate experimental chapters.

One of the aims of the research was to measure otoacoustic emissions (OAE) using recent technology to allow recording to lower stimulus levels than possible previously. Distortion product (DP) OAE were measured using a newly developed time averaging system and transient evoked (TE) OAE were measured using a maximum length sequence (MLS) technique. Both DPOAE and MLS TEOAE technology were used with the aim of recording lower level OAE than previously reported.

The methodology used in this thesis aimed to improve on previous studies published in the literature, firstly by improving the reliability of the OAE results. It had been suggested that DPOAE have limited usefulness as a result of DPOAE fine structure (Gorga et al, 1994). A new methodology was introduced to reduce the variability of DPOAE. This involved recording and averaging mini DP-grams around the octave frequencies to minimise the variability of the DPOAE fine structure (Hall and Lutman, 1999).

Both DP and TEOAE were recorded across the range of intensity levels, and were recorded down to and below the noise floor, with the objective of describing OAE growth-intensity functions (referred to as input-output or I/O functions).

TEOAE methodology was designed to record both the linear and nonlinear components of the OAE. This was different from many other studies that only recorded the nonlinear component of the OAE and subtracted out the linear component. This approach aided interpretation of OAE I/O functions.

All measurements were performed with the subject in a sound-treated booth and the researcher outside the booth. All measures were computer controlled, with the computer and audiometer located outside the booth. During OAE measures, subjects were instructed to keep as still as possible and to minimise swallowing.

All equipment was calibrated regularly, according to the calibration protocol described in Appendix 2.

4.2 CONVENTIONAL RATE TEOAE

4.2.1 Hardware

TEOAE were collected using the commercially available Otodynamics ILO288 Echoport, using software version 4.2. This is a PC based system, which operates in conjunction with the ILO Echoport. The probe used was the standard design ILO adult B-type probe containing a Knowles

BP1712 earphone and 1843 microphone (Kemp et al, 1990). A detailed account of the development and structure of the ILO is given in Bray (1989). Figure 4-1 shows a block diagram of the ILO288.

The Echoport generates and delivers the click stimuli and processes the resultant OAE. A crystal oscillator on the stimulus generation board of the Echoport triggers delivery of sets of four clicks, which are each 80 μs in length, generated digitally and delivered at a constant rate of 50 Hz. The clicks undergo digital-to-analogue conversion (DAC) before a low-pass filter removes any high frequency components introduced by the digital process. A digitally controlled attenuator alters the gain of the stimulus at this stage in steps of 1.5 dB if necessary, before transduction through the earphone of the probe.

The probe microphone records the response from the ear. The analogue output of the microphone is amplified using two amplifiers: a high-gain amplifier with a gain of 8500 amplifies the small signal OAE and a second low-gain amplifier with a gain of 400 amplifies the large, initial transient ear canal response. A low-pass filter avoids any aliasing artefacts in the processing. The response from each amplifier undergoes analogue-to-digital conversion with 12-bit resolution. The responses to each set of four clicks are sub-averaged, then alternate response waveforms are stored in A and B buffers where they are then averaged.

This digitised response is then further processed to extract the OAE. Fast Fourier transform (FFT) analysis of the OAE is performed by the PC. Prior to this, the data are high-pass filtered at 200 Hz to remove the tail of the click stimulus, known to contaminate the OAE response. The first 2.5 ms of the response, likely to be contaminated by stimulus, is zero-padded. Response arrays are then windowed using a function with a cosine-shaped rise and fall (rise and fall time of 2.56 ms) and a central plateau. After windowing, the response is digitally filtered between 0.6 and 6 kHz. The cross-power spectrum is then calculated using FFT analysis of the averaged A and B buffers. The TEOAE level is estimated from the real part of the cross-power spectrum, which contains those frequency components that are in-phase in the two buffers. This is converted to decibels and displayed by the ILO software as the RESPONSE measure.

4.2.2 Methods

The probe was coupled to the ear using foam tips supplied by Otodynamics. All recordings were made in the "linear mode" to ensure that the full TEOAE was recorded, including both the linear and nonlinear components of the OAE. As one of the aims of the project was to plot OAE I/O functions, it was important to record all components of the OAE. The cochlear amplifier (CA) is thought to operate linearly over low intensity ranges and it was essential that all aspects of this activity were recorded.

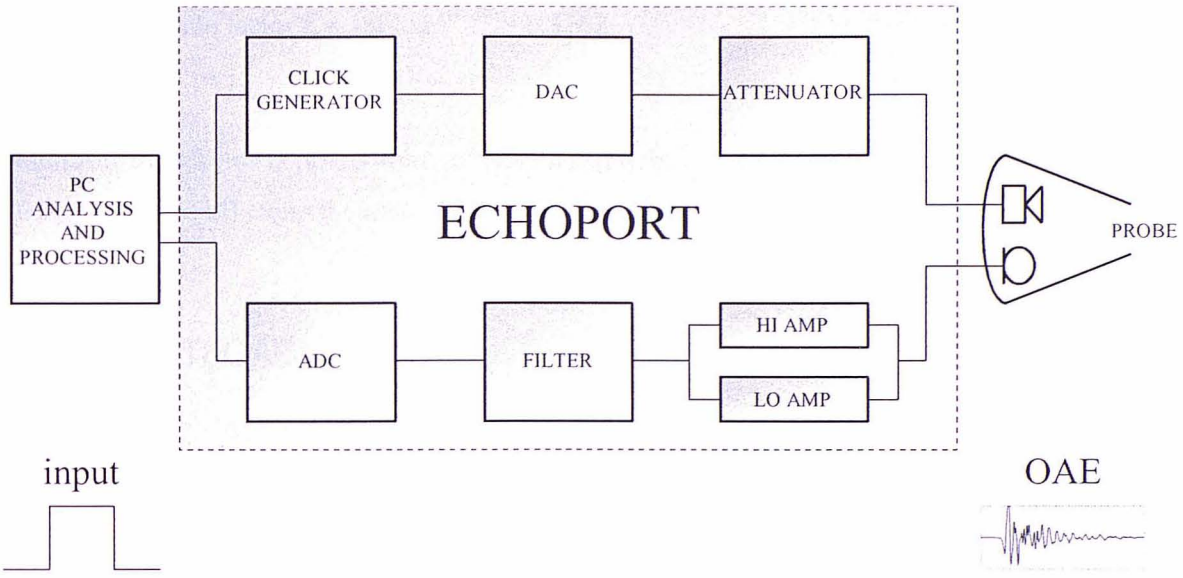


Figure 4-1: Block diagram of the ILO288 set-up (based on Bray, 1989). Key to abbreviations – ADC: analogue to digital converter, DAC: digital-to-analogue converter, HI AMP: high-gain amplifier, LO AMP: low-gain amplifier.

A time window of 20.44 ms and the conventional click rate of 50 clicks/s were used. Low-level stimulus levels have been shown to be more sensitive at detecting differences in cochlear function (Marshall and Heller, 1998). For this reason, TEOAE were obtained at stimulus levels of 90, 80, 70, 60, 50 and 40 dB SPL. This also allowed I/O functions to be plotted. These levels were set using gain values of +10.5, 0, –10, –19.5, –30 and –40.5 dB. Zero was equivalent to 80 dB SPL (see Calibration section, Appendix 2). Using the same gain settings for each subject ensured that a constant stimulus voltage was applied to the earphone for each subject. The noise rejection level was set individually for each subject at or below 44 dB if possible. Rejected sweeps were 10% or less of the total sweeps recorded; 260 sweeps (each containing four clicks) were routinely recorded for each subject giving an approximate recording time of one minute per stimulus level.

The ILO data were output in ASCII format. This allowed independent FFT analysis to verify the on-screen analysis of the ILO288 and also to allow derivation of the nonlinear waveforms. The FFT analysis is described in detail in Appendix 3. The nonlinear component of the TEOAE was derived from the linear TEOAE waveforms according to the method described by Kemp et al (1986). As only one waveform was recorded at each intensity level, it was necessary to re-scale the higher-level TEOAE to the lower-level TEOAE before subtraction, using the following formula:

Nonlinear at n dB: $(TEOAE_{n\ dB}) - (TEOAE_{n+10\ dB}/k)$

where the re-scale factor k = antilog $^{(10/20)}$ = 0.3162

Nonlinear waveforms at 60 dB were calculated using the mean of A and B waveforms at 60 and 70 dB, and at 70 dB using the mean of A and B waveforms at 70 and 80 dB.

4.3 MLS TEOAE

4.3.1 Hardware

MLS TEOAE were collected using equipment manufactured by Natus Medical Inc. The equipment was based on the Medical Research Council's Institute of Hearing Research system and was a prototype piece of equipment being developed, at the time of purchase, for hearing screening use in the USA and UK. Since then, the machine has been withdrawn for commercial reasons and is no longer available.

The Natus equipment comprised a PC containing a Loughborough Sound Image C31 DSP card, probe and pre-amplifier. The probe was an Otodynamics adult type-B probe identical to the model used by the ILO288 system. Figure 4-2 shows a block diagram of the equipment.

Stimuli are 100-μs unipolar clicks produced by an analogue click generator, triggered by pulses generated in the required sequence digitally on the DSP board. These range in maximum stimulation rate from 0.05 to 5 kHz. A digitally controlled attenuator alters the amplification of the stimulus in steps of 5 dB before transduction through the earphone of the probe.

The analogue output of the microphone is filtered between 0.5 to 5 kHz and sampled by the DSP board at a rate of 30 kHz. A 16-bit ADC recovers the sequences using the double buffer method described by Thornton et al (1994).

The digitised response is further processed by inspection of each recovered response to a sequence to check for excessive noise contamination, according to a user-set rejection template. Only recovered responses that meet the inclusion criteria are added to the ongoing averaging process. The algorithm used for on-the-fly recovery requires rapid processing by the DSP, as described by Thornton et al (1994).

Quality estimation of the OAE is also performed using the F_{sp} statistic. This estimates the ratio of the signal-to-noise (Elberling and Don, 1984; Lutman and Shepard, 1990). F_{sp} is calculated using the response waveform between 8 and 14 ms of the OAE for the numerator, and recording was terminated when the F_{sp} reached 500. Otherwise, recording continues until a pre-determined satisfactory number

of sequences are recorded. Processing of the signal, FFT analysis and subsequent calculations for detection of the OAE response are described in **Appendix 3**.

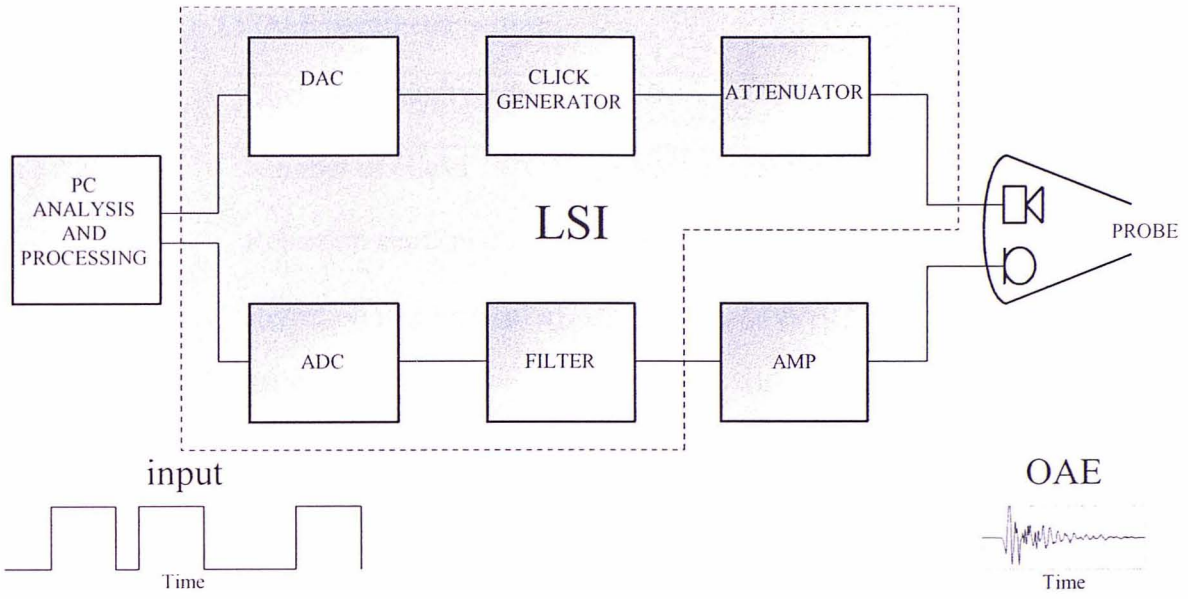


Figure 4-2: Block diagram of the MLS Natus experimental set-up. Key to abbreviations – ADC: analogue to digital converter, AMP: amplifier, DAC: digital-to-analogue converter, LSI: Loughborough Sound Images digital signal processing card.

4.3.2 Methods

The probe was coupled to the ear using foam tips supplied by Otodynamics. TEOAE were measured at click opportunity rates of 50, 500 and 5000 clicks/s. The click rates were chosen to include the conventional rate used in the ILO288 (50 clicks/s), and two higher rates covering the available range (500 and 5000 clicks/s). The linear TEOAE response was recorded. At each click opportunity rate, nominal stimulus levels of 80, 70, 60, 50 and 40 dB were used. A nominal level of 80 dB was equivalent to approximately 80 to 81 dB peak equivalent SPL (see Calibration section, **Appendix 2**). Using the same nominal setting for each subject ensured that a constant voltage was applied to the earphone for each subject. A 20 ms time window was used.

The recording time at each click rate was set to approximately one minute, so that it was equivalent to the recording time of the ILO288. The number of clicks presented therefore varied with click rate.

Noise rejection was pre-set for each click rate. Within the software, this is referred to as the noise rejection template. The rejection template was activated from 6 to 20 ms in the time window. The excess pressure settings (μPa) for rejection were determined by a pilot study, which derived the value

at which approximately 10% of sweeps were rejected. The full set of recording parameters used at each click rate is shown in Table 4-1.

Table 4-1: MLS TEOAE parameter settings

Click opportunity rate	50	500	5000
Number of clicks	800	5000	50000
Rejection start (ms)	6	6	6
Rejection pressure (μPa)	15000	10000	5000
MLS order	1	4	9

The data were output in ASCII format for FFT analysis as the MLS Natus software did not allow online FFT analysis (see **Appendix 3**). The nonlinear derived component of the MLS TEOAE data was calculated using the following formula:

$$((TEOAE_{n\text{-}10\,dB,\,1} + TEOAE_{n\text{-}10\,dB,\,2})/2) - (TEOAE_{n\,dB}/0.3162)$$

where $TEOAE_{n\text{-}10\,dB,\,1}$ was the first run at the lower stimulus level and $TEOAE_{n\text{-}10\,dB,\,2}$ was the second run at this level. The two runs were averaged before the subtraction process.

4.4 DPOAE

4.4.1 Hardware

DPOAE were collected using an in-house software and measurement system designed specifically for the purpose, developed jointly by the Medical Research Council's Institute of Hearing Research and the Institute of Sound and Vibration Research.

The equipment was a PC based system with a stand-alone ADC and DAC measurement unit, ER-10B probe-microphone and two Etymotic ER-2 insert earphones, Loughborough Sound Images C31 DSP card and custom software. Figure 4-3 shows a block diagram of the equipment.

Stimuli are pure tones generated digitally by the DSP card and DPOAE unit. The stimuli are converted to analogue form by the two 16-bit DAC. The pure tone waveforms are derived from sine

waves calculated and stored in memory buffers on the DSP card; each buffer of 2048 samples has a whole number of periods of both primaries. The sample rate is 32.768 kHz, giving a buffer duration of 62.5 ms. Hence, primaries can be generated at integer multiples of 16 Hz. The software alters the level of the stimulus waveform in 1 dB steps, with a range of 10 to 80 dB SPL. Stimuli are transduced through ER-2 insert earphones, selected because of their flat frequency response.

The ear canal sound pressure is recorded by an Etymotic ER10-B probe microphone and pre-amplifier, sampled by custom software running on the DSP card, also at a sampling rate of 32.768 kHz and a buffer size of 2048. This gives a frequency resolution of 16 Hz. Immediately on completion of each buffer acquisition, the DSP card transforms the waveform into the frequency domain and the complex FFT is transferred to the PC for further processing.

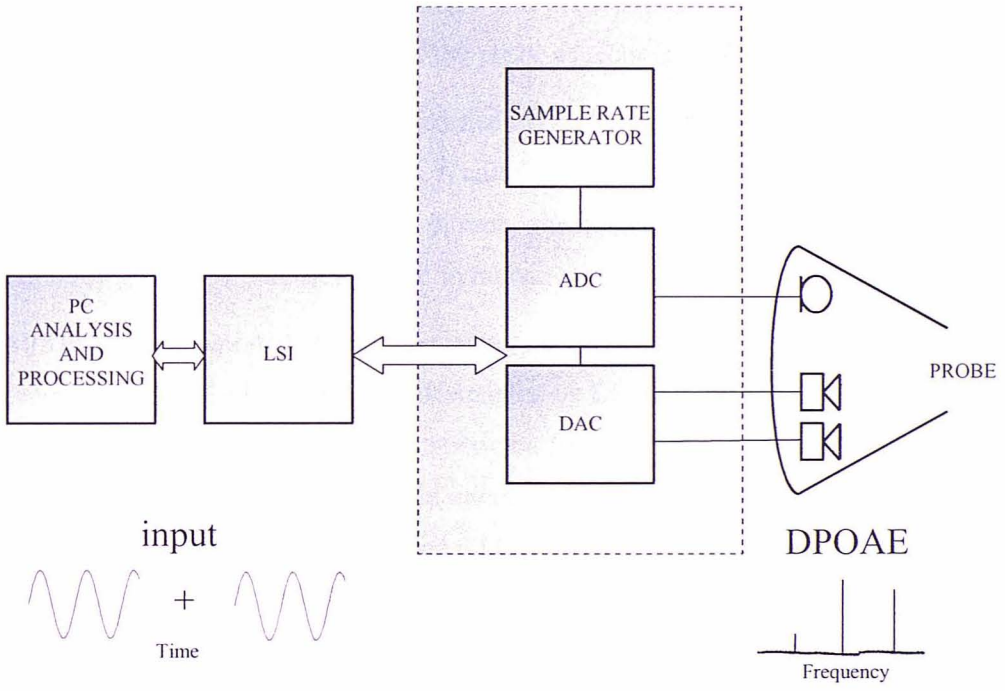


Figure 4-3: Block diagram of the DPOAE experimental set-up. Key to abbreviations – ADC: analogue to digital converter, DAC: digital-to-analogue converter, LSI: Loughborough Sound Images digital signal processing card.

Software running on the PC examines each FFT buffer and calculates the noise level in the region of the DP frequency by averaging the power in the 10 frequency components either side of the DP frequency (i.e. 20 in all). Buffers are rejected if this average noise level exceeded a user-set criterion. Accepted buffers are included in the complex averaging of the FFT. This approach allows more selective rejection of noisy sweeps than systems that rely on time-domain averaging where the time-domain waveform is dominated by the primary stimuli. As with time-domain averaging, complex FFT

averaging has the benefit of improving the signal-to-noise ratio of the response with successive buffers. Systems that use a spectrum analyser, which does not consider the phase of each frequency component, do not improve SNR with averaging but simply smooth the response. This system of complex averaging and rejection, based on frequency-domain analysis, has the advantage over many other recording systems in that it allows DPOAE at smaller levels to be recorded because the noise floor is typically below –25 dB SPL per 16-Hz band, although it can be as low as –40 dB with the parameters used here. After each buffer is added to the average, the SNR of the DP estimate is calculated from the averaged magnitude of the FFT component at the DP frequency and the averaged magnitude of the 20 components used for noise estimation. The availability of this ongoing SNR estimate allowed the averaging to be stopped as soon as a user-set SNR had been reached.

4.4.2 Methods

The probe was coupled to the ear using a soft plastic tip. The earphone sound delivery tubes projected approximately 5 mm from the tip of the probe as recommended by the manufacturer.

The distortion product $2f_1-f_2$ was recorded in this study. DPOAE were elicited by stimulating with two continuous pure-tone primaries, f_1 and f_2 ($f_1<f_2$). The primary tone at f_1 was delivered via one earphone and f_2 via the other. The f_2/f_1 ratio was fixed at 1.2 for all testing, as ratios close to this generally give the strongest DPOAE in human ears (Probst et al, 1991).

All DPOAE are reported with reference to f_2 as this frequency is understood to be closest to the $2f_1-f_2$ generation site. The level of f_1 was designated by L1 and f_2 by L2. L1 was always 10 dB above L2. Mini DP-grams were recorded over restricted frequency ranges of 1.9-2.1, 2.9-3.1, 3.9-4.1 and 5.9-6.1 kHz referenced to f_2, using 48 Hz intervals. The ranges were centred on the conventional audiometric frequencies of 2, 3, 4 and 6 kHz. It is expected that the average response from the five adjacent frequencies of the mini DP-gram is less idiosyncratic than DPOAE recorded at one frequency point only. It was considered that averaging the mini DP-gram reduces the variability caused by DP fine structure and reduces the errors that can arise if a DPOAE is recorded lying close to a peak or trough of the fine structure.

Mini DP-grams were measured at seven L1/L2 levels: 20/10, 30/20, 40/30, 50/40, 60/50, 70/60 and 80/70 dB SPL Using the same nominal setting for each subject ensured that a constant voltage was applied to the earphone for each subject. See calibration section in Appendix 2 for further details.

The averaging capabilities of the DPOAE equipment enabled recordings to be made with a low noise level, thus allowing recordings to be made with lower stimulus levels than previous studies. Low stimulus intensity levels have been reported to be more sensitive to changes in cochlear function (Gaskill and Brown, 1990), so it was important to be able to obtain responses over the entire range of levels.

The noise rejection was set within the software so that sweeps with components at frequencies adjacent to the DP frequency exceeding 10 dB SPL were discarded. Recording terminated at an estimated SNR of 15 dB after a minimum of 20 stimulus epochs, or alternatively after a maximum of 100 accepted sweeps, whichever condition was met first.

4.5 SPONTANEOUS OTOACOUSTIC EMISSIONS

A check for the presence of spontaneous (S) OAE was carried out in Experiment 2 for each subject as large SOAE can affect the measurement of other OAE types.

SOAE were recorded using the same equipment as DPOAE by recording for 100 sweeps in the absence of any stimulus, averaging the power spectrum across sweeps. Rejection of noise buffers was based on noise level estimated from 20 frequency components in the vicinity of 1 kHz. This frequency was chosen arbitrarily.

SOAE were deemed to be present when the level at a particular frequency bin was 5 dB above the noise floor. 5 dB rather than 3 dB was used to avoid accepting erroneous responses, as the equipment was not designed specifically to measure SOAE. The SOAE also had to be present on two repeats on two separate days.

4.6 AUDIOMETRY

4.6.1 Hardware

Audiometry was performed using an in-house software and measurement system, run from a 16-bit sound card housed in a PC, through an audiometer and TDH-39 supra-aural earphones. A 40-dB 10-ohm attenuator was used between the audiometer and the earphones to minimise low level background noise introduced by the sound card.

The stimuli were 200-ms tone bursts, generated digitally with onset and offset cosine ramps, delivered at a rate of 2.5 per second. Frequency was changed in 50 Hz steps. Level was changed by 2 dB every 2 tone bursts either up or down, according to whether the subject response button was pressed.

4.6.2 Methods

Hearing threshold levels were measured using a swept-frequency self-recording audiometric technique. Audiometric fine structure was recorded around the conventional audiometric frequencies for the same reasons as for DPOAE. Threshold was recorded over a restricted frequency range of 0.9-1.1, 1.9-2.1, 2.9-3.1, 3.9-4.1 and 5.9-6.1 kHz using 50 Hz intervals. Averaging over this frequency range is predicted to reduce the influence of fine structure.

To improve measurement accuracy, two repeats were obtained at each frequency block and the mean result taken across these two repeats. Recording was made in the six separate frequency blocks using

a randomised design to vary the order of blocks between subjects. The initial test ear was alternated between test sessions for each subject.

All results are reported in dB HL. For further details see calibration section in Appendix 2

PART 2

EXPERIMENTAL STUDIES

This thesis aims to evaluate the relationship between OAE and HTL with particular reference to mild sensory hearing loss.

Firstly an investigation is made of OAE from normal hearing subjects. This describes the variation between subjects within the normal hearing group. This is then extended to investigate the cross-sectional differences in OAE in subjects with a range of HTL up to mild/moderate hearing impairment.

An investigation of longitudinal changes in OAE is made in subjects developing a mild, temporary sensory hearing impairment.

A comparison is made between cross-sectional differences with longitudinal changes in both DP and TEOAE. For a meaningful comparison, it was important to perform both the cross-sectional and longitudinal experiments using the same equipment and methodological parameters.

All experiments were approved by the Institute of Sound and Vibration Research Human Experimentation Safety and Ethical Committee. Informed consent to participate in the study was obtained from each subject.

5 EXPERIMENT 1: CROSS-SECTIONAL STUDY

5.1 AIMS

Experiment 1 aimed to investigate measures of otoacoustic emissions (OAE) in human subjects, and to explore the relationship between OAE and hearing threshold level (HTL) through examination of differences in OAE between subjects with differing HTL (normal to mild/moderate sensory hearing losses).

5.2 OBJECTIVES

The main objectives of Experiment 1 were:

To describe the normative properties of DP, TE and MLS TEOAE, using the methodology described in Part 1. Also to describe the variation of DP, TE and MLS TEOAE in subjects with a range of HTL.

To explore measures of OAE in subjects with a range of HTL.

To derive a baseline relationship between OAE and HTL, for comparison with the longitudinal results of Experiment 2.

To establish the short- and medium-term repeatability of OAE measures.

5.3 HYPOTHESES

This thesis is based on the assumption that OAE and HTL have a close relationship because OAE reflects outer hair cell (OHC) function, and mild sensorineural hearing impairment is predominantly due to OHC loss. The alternative to this assumption is that other factors are also important for both HTL and OAE.

Experiment 1 tests the hypothesis that the moderate correlations of previous studies are a result of a poor choice of OAE measures. It is postulated that the underlying relationship between OAE and HTL is close, but is not detectable as a result of the OAE measures used in previous studies. This hypothesis will be tested by investigation of a wide range of OAE measures based on simple models, using a range of stimulus levels and stimulus rates. The relationship of these OAE measures and HTL will be examined. It is expected that OAE measures that account for the level dependency of the CA have a higher correlation with HTL than other measures.

Additionally, specific hypotheses tested are that OAE I/O functions from human subjects with a range of HTL will reflect the framework of OAE I/O functions outlined in Part 1.

1. Differences in HTL will be reflected by differences in:

    a. OAE I/O function nonlinearity

b. OAE level (at a pre-set stimulus level)

c. OAE stimulus level (at a pre-set OAE level)

2. OAE measures will reflect differences in HTL. Specifically,

   a. OAE level (at a pre-set stimulus level) will be negatively correlated with HTL, with decreasing OAE level associated with increasing HTL

   b. OAE stimulus level (for a pre-set OAE level) will be positively correlated with HTL, with increasing OAE stimulus level associated with increasing HTL

   c. MLS rate suppression will be negatively correlated to HTL, with decreasing rate suppression associated with increasing HTL

3. TEOAE measures will have a higher correlation with HTL than DPOAE measures, due to the primarily reflection source generation mechanism of TEOAE (Shera and Guinan, 1999).

4. OAE measured using new technology will show a higher correlation with HTL than previous studies. Specifically:

   a. DPOAE will be measured at lower level than previously possible, and will thus have a higher correlation with HTL than published studies

   b. MLS TEOAE will be recorded at lower level than conventional TEOAE and will thus have a higher correlation with HTL than conventional TEOAE

   c. Consideration of cochlear fine structure will result in more repeatable and more easily interpreted measures of DPOAE and hence improve the relationship to HTL

5.4 METHODOLOGY

This experiment used a cross-sectional design to investigate differences in OAE between subjects. Repeated measures enabled repeatability of OAE and HTL to be estimated.

5.4.1 Subjects

Subjects were recruited from the university staff and student population, and were selected based on their hearing thresholds. A range of HTL were required, from normal hearing to moderate sensorineural hearing impairment. Hearing loss was required to be age-related, with no other significant audiological history. Five subjects were required per HTL group, based on the 3-6 kHz average HTL and ranging from < 0 dB to > 40 dB in 10 dB steps.

All subjects had normal hearing or a mild to moderate bilateral sensorineural hearing impairment, with bone conduction audiometric thresholds within 5 dB of air conduction values. All subjects had

normal middle-ear function as determined by otoscopy and tympanometry (middle-ear compliance between 0.3 to 1.5 ml, middle-ear pressure between –50 and +50 daPa). Any other contra-indications to participation in the study were determined by questionnaire, see **Appendix 4**.

Forty-three subjects were tested, 21 males and 22 females, ranging in age from 21 to 73 years. One ear only of each subject was tested due to time constraints. Left and right ears were alternated between subjects unless contraindicated by the exclusion criteria above.

5.4.2 Measures

All measures were obtained according to the methodology described in Chapter **4**. DPOAE measures were restricted to 3 kHz and above, as these were the frequencies at which there were the largest differences in HTL between subjects. Audiometry was also restricted to the range from 3 to 6 kHz.

Each subject was tested four times in total. The first two sessions were performed within a maximum of 8 weeks of each other, with an interval of at least 24 hours. The second two sessions were performed 9 months later, separated by an interval of at least 24 hours and a maximum of 9 weeks. This enabled both short-term and medium-term repeatability to be estimated.

OAE measures were obtained according to the methodology described in Chapter **3**.

5.5 NORMATIVE STUDY

This section describes the characteristics of OAE obtained from normal hearing ears. The normative properties of TEOAE recorded at the conventional click rate are described. Normative properties of MLS TEOAE and DPOAE are also described.

5.5.1 Subjects

Eighteen of the subjects from the cross-sectional study, with hearing threshold less than or equal to 20 dB HL at 0.25-8 kHz, were included in the normative group (ten female, eight male). The mean age of the subjects was 38 years (range 21 to 59 years), and the mean audiogram of the group is shown in Figure 5-1. This included 5 left ears and 13 right ears.

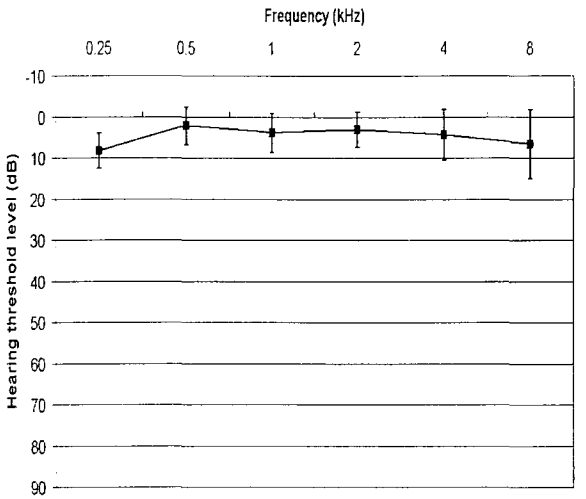


Figure 5-1: Mean hearing threshold levels (± 1 standard deviation) of the normative subject group.

5.5.2 Conventional rate TEOAE

TEOAE were recorded from two different instruments, the ILO288 and the MLS Natus system. The ILO288 uses conventional recording methods and the Natus system uses both conventional recording methods and the maximum length sequence technique. The properties of TEOAE from the ILO288 are first described in detail.

5.5.2.1 Linear waveforms

TEOAE are complex time domain waveforms composed of a number of frequency components. They are unique to individual ears, highly repeatable within subjects, and show large variation between subjects.

The ILO288 has a 20.44 ms time window and TEOAE waveforms from the ILO288 are plotted from 0.92 to 20.44 ms. During recording, half the TEOAE responses are stored in buffer A and half in buffer B. The ILO288 plots both the A and B waveforms, thus allowing estimation of waveform repeatability. Each TEOAE has a characteristic shape, which is highly repeatable within subjects but very different for each individual. Example waveforms from two different subjects are shown in Figure 5-2.

Figure 5-3 shows TEOAE waveforms recorded from the same subject at different click intensity levels. This shows a reduction in level with decreasing stimulus level, but similar waveform morphology at each stimulus level. The waveform component before 5 ms disappears with decreasing stimulus level, and is probably either stimulus artefact or a linear component. Stimulus level does not materially affect the morphology of the waveform, only the TEOAE level.

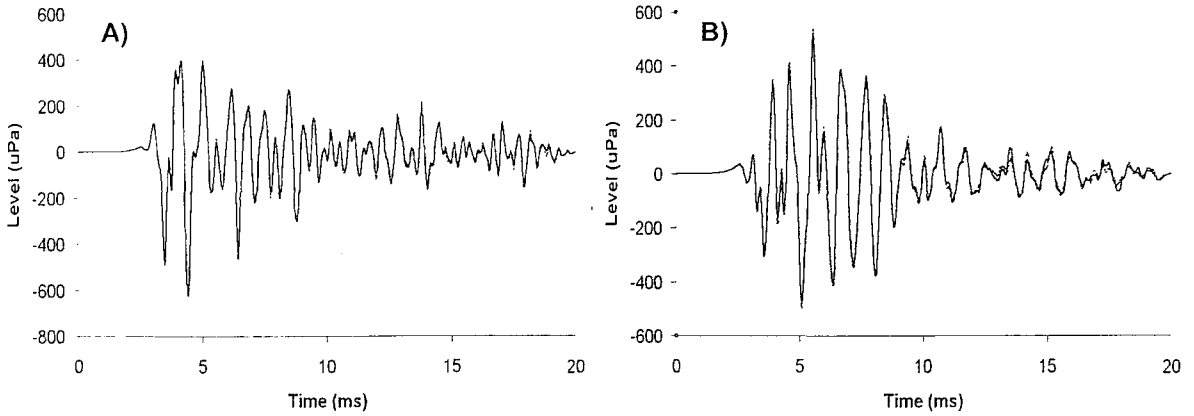


Figure 5-2: Linear TEOAE waveforms evoked by 80 dB click stimuli for A) Subject 5 and B) Subject 28. Waveforms obtained from different subjects have different morphology, which are highly repeatable within individuals. Key: wave A: solid line, wave B: dashed line.

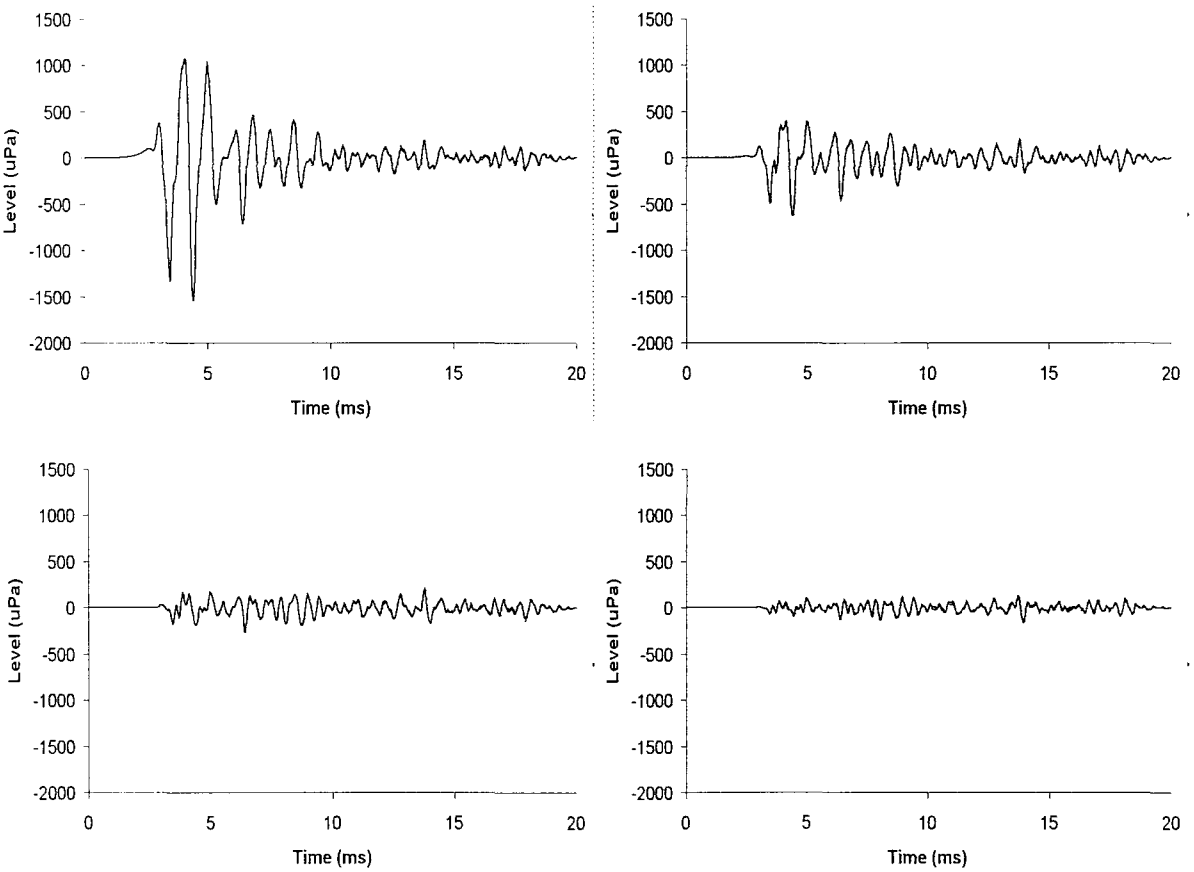


Figure 5-3: Examples of linear TEOAE waveforms evoked by different stimulus levels from subject 5 with A) 90 dB click level, B) 80 dB click level, C) 70 dB click level and D) 60 dB click level. There is a reduction in waveform level with decreasing stimulus level, but the waveform morphology is maintained.

The A and B waveform data were used to calculate the waveform repeatability within-session. This was calculated by measuring the cross-correlation coefficient of the A and B waveforms within each session[2]. Repeatability was measured for the entire 20 ms waveform and also for different time divisions of the A and B waveforms, at each stimulus level.

Table 5-1 gives the mean cross-correlation coefficients of the TEOAE waveforms obtained from the normal hearing subjects. This showed an increase in repeatability of the waveform with increasing waveform latency up to approximately 9 ms. After 9 ms there was a reduction in repeatability, consistent with a smaller signal component relative to the noise. Repeatability of the waveform also increased with increasing stimulus level.

Table 5-1: Mean (SD) cross-correlation coefficients of the linear TEOAE A versus B waveforms within-session

Time (ms)	Click level (dB SPL)					
	40	50	60	70	80	90
1-20	0.45	0.70	0.78	0.90	0.98	0.99
	(0.21)	(0.24)	(0.25)	(0.14)	(0.02)	(0.00)
5.03-5.99	0.45	0.80	0.84	0.87	0.99	0.99
	(0.36)	(0.17)	(0.27)	(0.25)	(0.01)	(0.00)
6.03-6.99	0.59	0.81	0.88	0.91	0.98	0.99
	(0.35)	(0.22)	(0.21)	(0.23)	(0.04)	(0.00)
7.03-7.98	0.60	0.74	0.83	0.90	0.94	0.99
	(0.32)	(0.29)	(0.25)	(0.21)	(0.27)	(0.02)
8.02-8.98	0.55	0.73	0.83	0.90	0.97	0.97
	(0.39)	(0.36)	(0.31)	(0.23)	(0.05)	(0.08)
9.02-12.97	0.46	0.72	0.81	0.90	0.93	0.97
	(0.33)	(0.28)	(0.28)	(0.18)	(0.16)	(0.05)
13.02-16.97	0.40	0.67	0.79	0.84	0.87	0.90
	(0.25)	(0.28)	(0.24)	(0.26)	(0.24)	(0.12)

The between-session repeatability of the waveforms was assessed over sessions 3 and 4. Cross-correlation coefficients of the TEOAE waveforms between sessions 3 and 4 were calculated. The

[2] Within the ILO288 software, this measure of repeatability is known as reproducibility.

mean group results are shown in Table 5-2. This showed an increase in repeatability with increasing stimulus level, up to a stimulus level of 80 dB, and then a decrease in repeatability at 90 dB. The early latency sections of the waveform were slightly more repeatable than the later sections. The between-session repeatability is lower than the repeatability within-session.

Table 5-2 Mean (SD) cross-correlation coefficients of the linear TEOAE waveforms between sessions 3 and 4

Time (ms)	Click level (dB SPL)					
	40	50	60	70	80	90
1-20	0.54	0.64	0.74	0.72	0.71	0.62
	(0.24)	(0.35)	(0.30)	(0.33)	(0.30)	(0.34)
5.03-5.99	0.53	0.63	0.75	0.77	0.76	0.67
	(0.28)	(0.34)	(0.28)	(0.26)	(0.30)	(0.37)
6.03-6.99	0.67	0.76	0.78	0.78	0.76	0.63
	(0.22)	(0.23)	(0.34)	(0.30)	(0.30)	(0.43)
7.03-7.98	0.58	0.61	0.79	0.80	0.79	0.75
	(0.37)	(0.47)	(0.23)	(0.26)	(0.30)	(0.32)
8.02-8.98	0.64	0.60	0.75	0.75	0.71	0.72
	(0.25)	(0.46)	(0.37)	(0.40)	(0.53)	(0.46)
9.02-12.97	0.59	0.62	0.75	0.74	0.79	0.76
	(0.22)	(0.36)	(0.30)	(0.33)	(0.24)	(0.24)
13.02-16.97	0.35	0.52	0.67	0.65	0.68	0.67
	(0.37)	(0.44)	(0.37)	(0.46)	(0.41)	(0.43)

5.5.2.2 Derived nonlinear waveforms

A recording method that is commonly used within the ILO288 is the "derived nonlinear" method. In this method, the linear components of the waveform are subtracted, leaving only the nonlinear components of the waveform.

In this study, waveforms were recorded in the "linear" rather than the nonlinear mode because it was considered important to record the entire waveform without losing any components, whether linear or nonlinear. From the linear waveforms it was possible to derive the nonlinear waveforms, and therefore allowed comparison of the results from the two recording methods.

The nonlinear components of the waveforms were derived from the linear waveforms as described in Section 4.2.2 and further analysed. Figure 5-4 compares linear and derived nonlinear waveforms from the same subject.

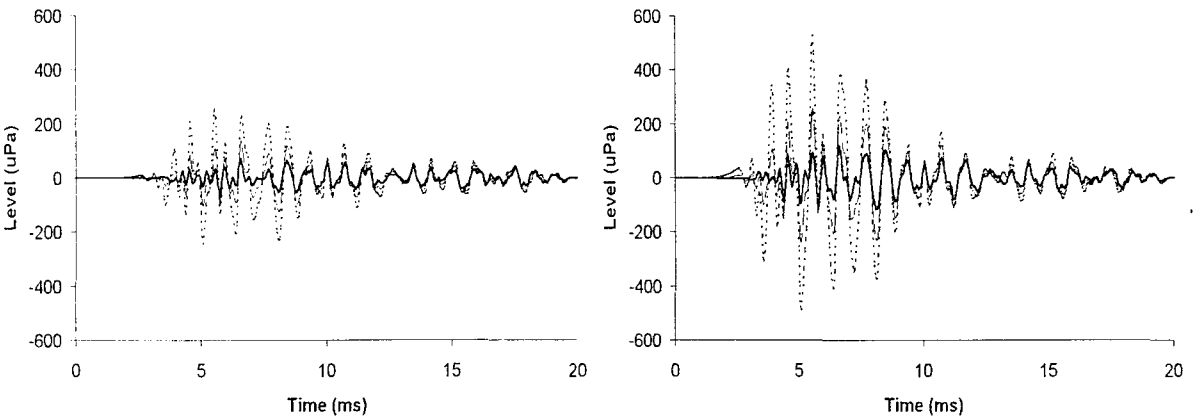


Figure 5-4: Examples of derived nonlinear waveforms from subject 28 at A) 60 dB click level and B) 70 dB click level. Key: thick line: derived nonlinear waveform, thin solid and dashed lines: linear waveforms. Note: The 60 dB derived nonlinear waveform was derived from linear waveforms evoked by 60 and 70 dB click stimuli; 70 dB was derived from linear waveforms evoked by 70 and 80 dB click stimuli.

Figure 5-4 shows that the level of the derived nonlinear waveform is smaller than the level of the linear waveforms. This also shows that the main linear sections of the waveform occur within the first 10 ms of the waveform. From 10 to 20 ms, most of the waveform is nonlinear, and little linear subtraction is required.

The repeatability of the derived nonlinear waveform within-sessions was not calculated, as only one waveform at each level was derived per session. However repeatability of the nonlinear derived waveform was assessed between sessions 3 and 4. This was measured by calculating the cross-correlation coefficient of the nonlinear derived waveform over sessions 3 and 4, for each subject at 60 and 70 dB. The mean group results are shown in Table 5-3.

Table 5-3 Mean (SD) cross-correlation coefficients of the nonlinear TEOAE waveforms between sessions 3 and 4

Time (ms)	Click level (dB SPL)	
	60	70
1-20	0.65	0.65
	(0.32)	(0.35)
5.03-5.99	0.61	0.71
	(0.35)	(0.28)
6.03-6.99	0.73	0.65
	(0.32)	(0.43)
7.03-7.98	0.69	0.74
	(0.31)	(0.25)
8.02-8.98	0.72	0.68
	(0.28)	(0.47)
9.02-12.97	0.66	0.69
	(0.32)	(0.35)
13.02-16.97	0.54	0.61
	(0.34)	(0.42)

Note: The 60 dB derived nonlinear waveform was derived from linear waveforms evoked by 60 and 70 dB click stimuli; 70 dB was derived from linear waveforms evoked by 70 and 80 dB click stimuli.

The between-session repeatability of the derived nonlinear waveform was similar at click levels of 60 and 70 dB. The highest repeatability was measured for the mid-latency waveform components. Comparison of the between-session repeatability of the derived nonlinear waveforms with the linear waveforms showed poorer repeatability of the nonlinear waveforms.

5.5.2.3 Frequency response

The linear and the derived nonlinear waveform data were analysed using fast Fourier transform (FFT) analysis (see **Appendix 3** for method). This calculates the relative components of the TEOAE waveform at each frequency. Figure 5-5 shows examples of FFT responses from two subjects of linear waveforms evoked by an 80 dB click stimulus.

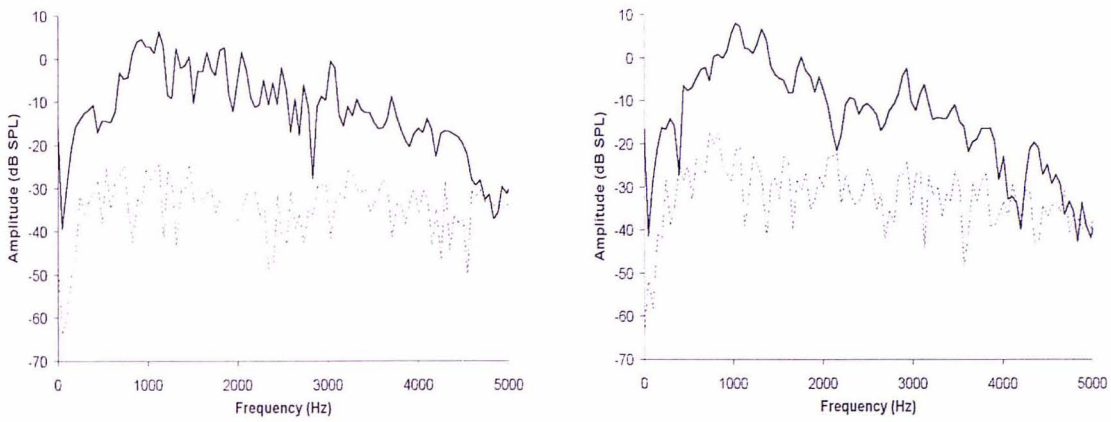


Figure 5-5: Example frequency response of linear TEOAE waveforms evoked by 80 dB click level for A) Subject 5 and B) Subject 28. Key: Solid line: TEOAE response; broken line: noise floor.

The FFT responses of all subjects were averaged. The mean TEOAE FFT responses of the normal hearing subjects at each stimulus level, for the linear waveforms are shown in Figure 5-6.

Within the TEOAE FFT, energy was concentrated mostly at 1 kHz, reducing in level with increasing frequency. As stimulus level was reduced, the greatest reduction in energy occurred at 1 kHz. The upper frequency limit at which TEOAE level was greater than the noise floor was approximately 5 kHz at the higher stimulus levels, and approximately 4 kHz at a stimulus level of 50 dB.

Figure 5-7 compares the FFT of the linear and derived nonlinear TEOAE waveforms. This showed similar frequency responses, although the level of the nonlinear FFT was smaller than the linear FFT across the frequency range.

5.5.2.4 Input-output functions

From the FFT response, the levels of the TEOAE in individual frequency bands were calculated. Plotting the level response as a function of stimulus level is known as an input-output (I/O) function.

The level of the linear and nonlinear TEOAE response in individual frequency bands was calculated and TEOAE I/O functions were plotted at each frequency. Noisy data were excluded from the analysis. Individual I/O functions from linear and nonlinear waveforms are plotted in Figure 5-8. Mean TEOAE level ± 1 SD was calculated at each frequency. The results are plotted in Figure 5-9. These showed a reduction in TEOAE level with increasing frequency. There was also an increase in compression with increasing frequency. The morphology of the I/O functions was similar for both the linear and nonlinear responses, although the nonlinear functions were lower in level.

The slope of each I/O function was estimated with linear regression analysis, using the level points evoked to click levels of 60-80 dB. Table 5-4 summarises the median and percentiles of I/O function slope values for the linear and nonlinear TEOAE I/O functions.

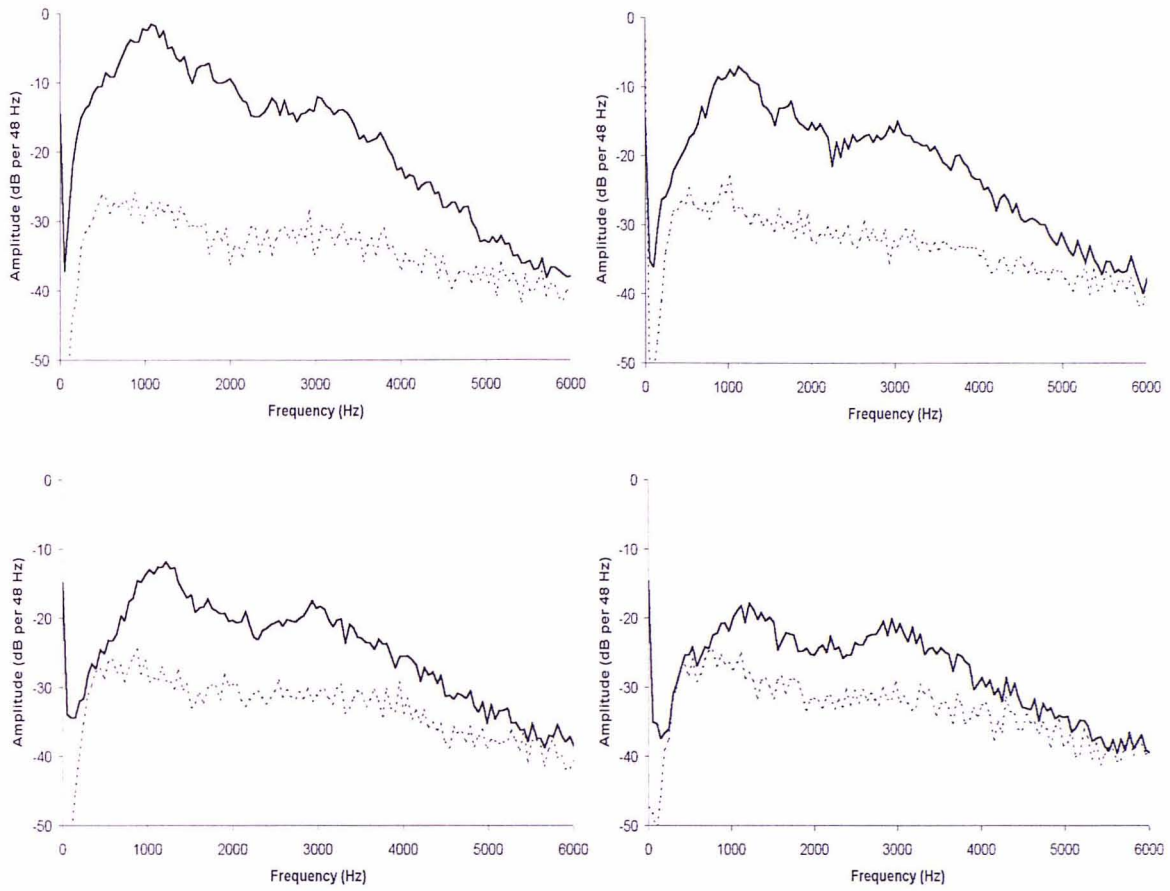


Figure 5-6: Mean FFT of linear TEOAE waveforms evoked by different stimulus levels, from normal hearing subjects at A) 80 dB click level, B) 70 dB click level, C) 60 dB click level and D) 50 dB click level. Key solid line: FFT of TEOAE waveform, dashed line: FFT of noise floor.

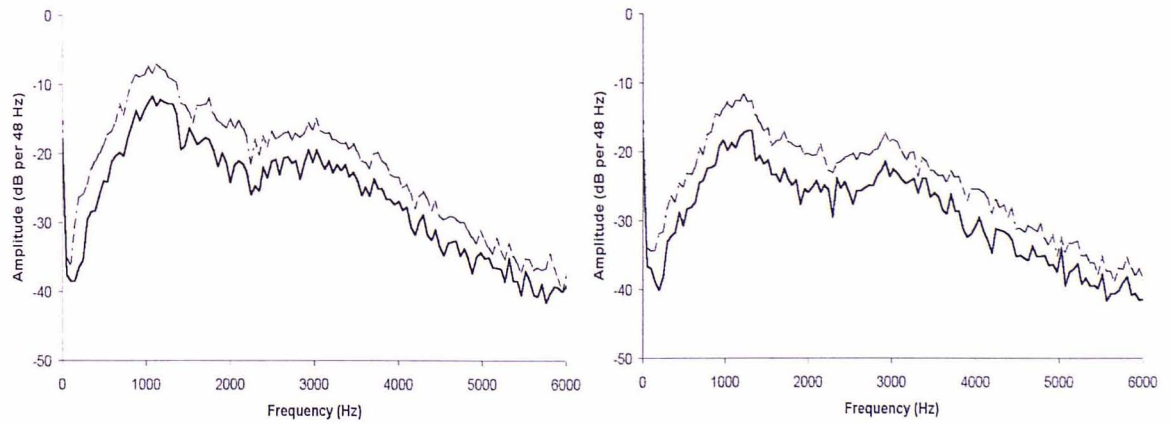


Figure 5-7: Mean FFT comparing linear and nonlinear TEOAE waveforms evoked by different stimulus levels, from normal hearing subjects at A) 70 dB click level and B) 60 dB click level. Key Dashed line: FFT of linear waveforms, solid line: FFT of nonlinear waveforms.

Table 5-4 Median and percentile slope values of linear and nonlinear TEOAE I/O functions (dB/dB)

Recording method	Percentile	Frequency (kHz)				
		1	2	3	4	BB
Linear	25th	0.34	0.38	0.21	0.23	0.33
	Median	0.62	0.44	0.31	0.29	0.50
	75th	0.74	0.55	0.36	0.32	0.58
Nonlinear derived	25th	0.41	0.21	0.22	0.12	0.26
	Median	0.54	0.44	0.29	0.24	0.36
	75th	0.64	0.53	0.39	0.39	0.47

Key BB: broadband

A slope of 1.0 corresponds to a 1 dB/dB relationship between OAE level and stimulus intensity. Table 5-4 shows that at each frequency, and for the broadband response, the relationship between OAE level and stimulus has a slope less than unity, showing a saturating nonlinear relationship. There was an increase in compression with increasing frequency, and the variation in compression between subjects decreased with increasing frequency. The slope values were similar for the linear and nonlinear I/O functions at all frequencies except the broadband response. The nonlinear broadband I/O function has a shallower slope than the equivalent linear function.

5.5.2.5 Repeatability

Any test has associated test-retest repeatability. If a test is performed on two occasions with no change in the subject under test, it is unlikely that exactly the same result will be obtained both times, solely due to test-retest uncertainty. The greater the repeatability, the smaller the expected difference in the results of the two tests.

One indicator of repeatability is the standard deviation (SD) of the difference on replication. The SD of the difference on replication incorporates the accumulated uncertainty of the two measurements. If each replication has the same uncertainty (within-subject variance), the difference has double the variance. Hence the within-subject SD of each measure can be estimated by dividing the SD of the difference on replication by $\sqrt{2}$. This is termed the *replication SD*. Throughout this thesis, repeatability is expressed in terms of the replication SD estimated in this way.

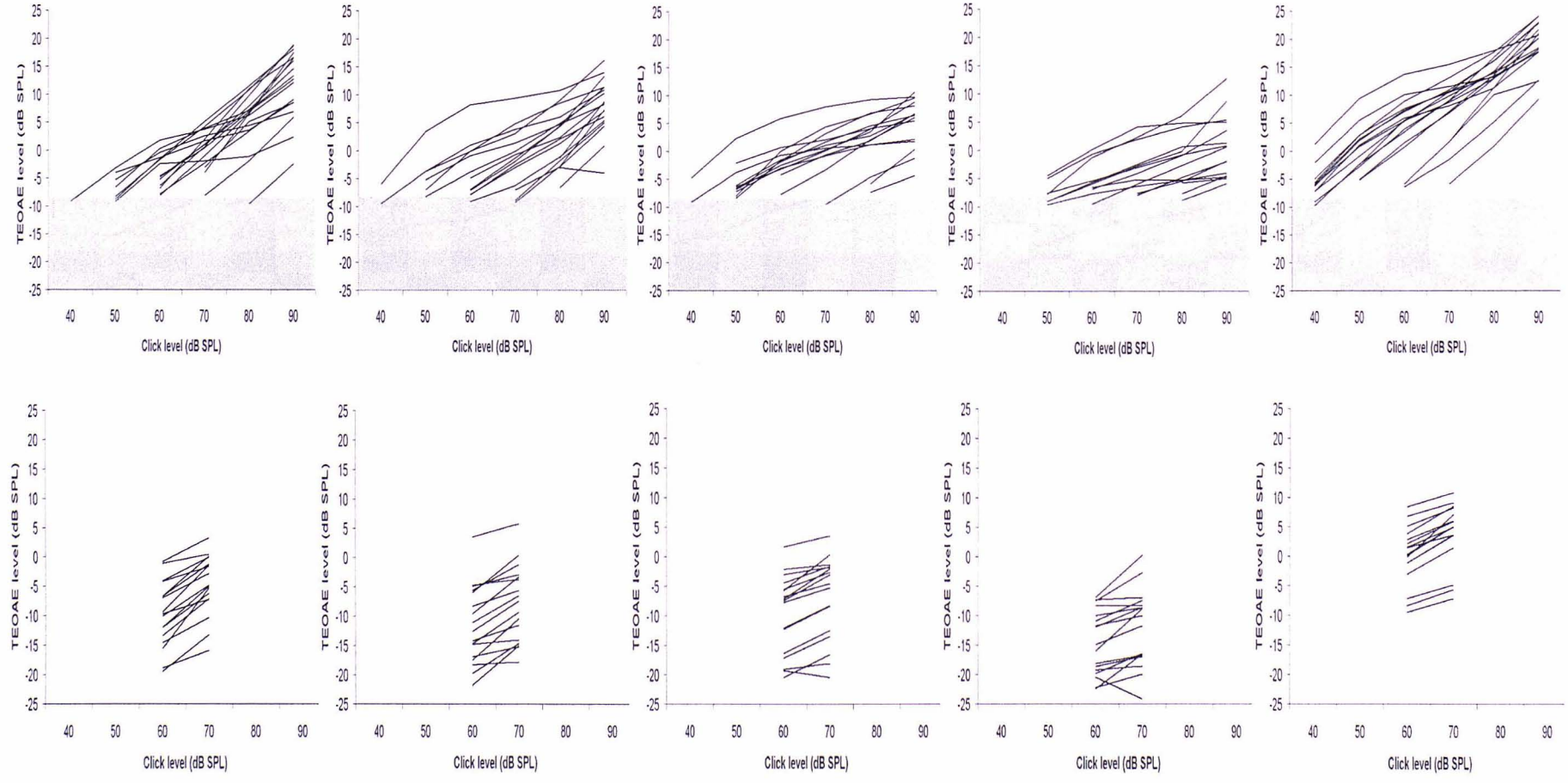


Figure 5-8: TEOAE I/O functions from normal hearing subjects plotted from linear (top line) and derived nonlinear waveforms (bottom line). The nonlinear I/O functions were measured at 60 and 70 dB only. Key to abbreviation – BB: broadband. Mean noise floor shown by shaded area.

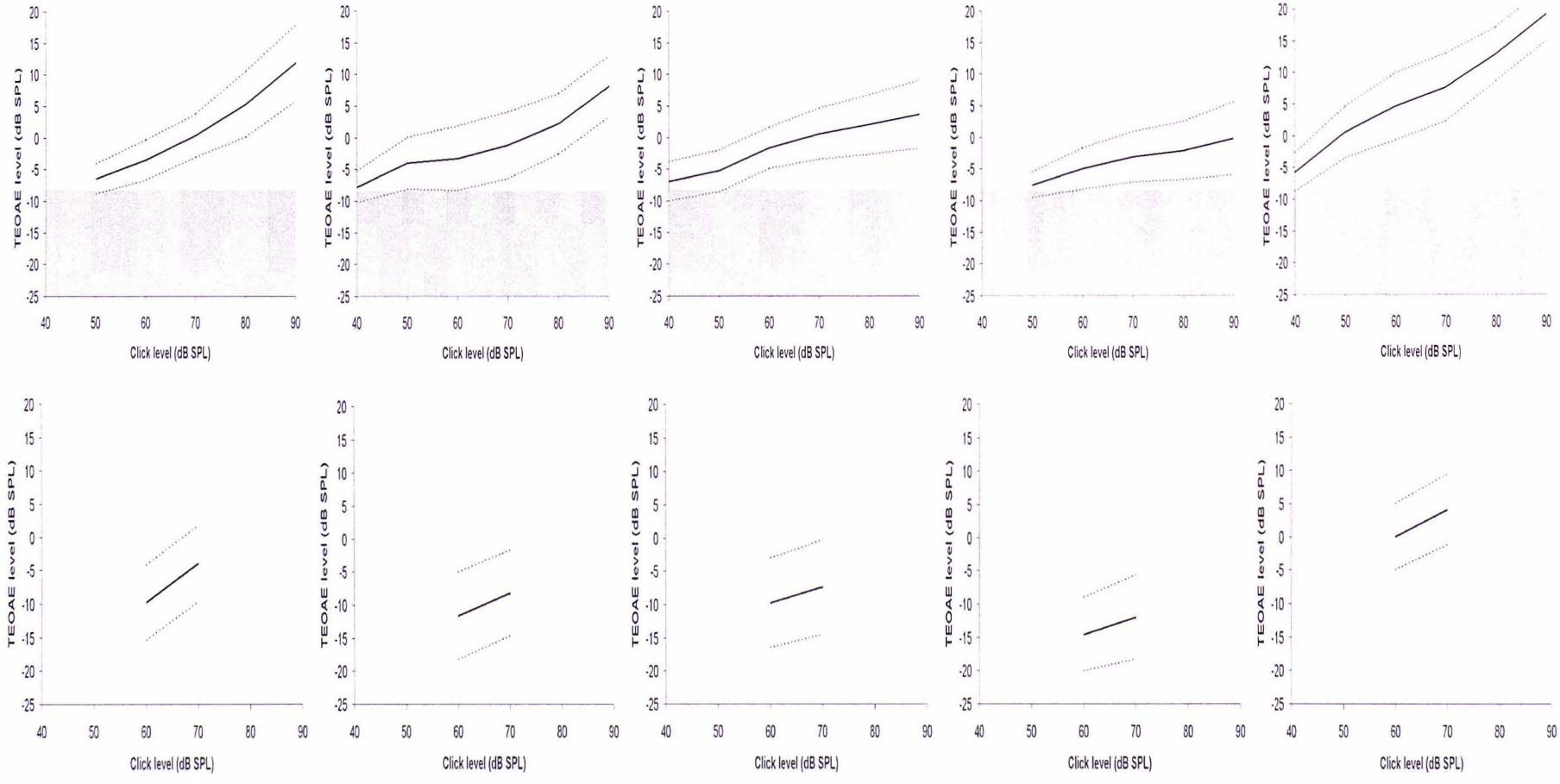


Figure 5-9: Mean TEOAE I/O functions (solid line) ± 1 standard deviation (dashed lines) from normal hearing subjects plotted from linear and derived nonlinear waveforms. Key to abbreviation - BB: broadband. Mean noise floor shown by shaded area.

Test-retest repeatability of TEOAE level was calculated by estimating the replication SD for each measure. Analysis was performed for each linear and nonlinear TEOAE measure. Replication SD was calculated using all normally hearing subjects. Short-term repeatability was calculated using the results of sessions 3 and 4 recorded within one month of each other. Medium-term repeatability was calculated using the mean results of session 1 and 2, and compared with the mean results of sessions 3 and 4 recorded at least 9 months later. Results of short- and medium-term repeatability are shown in Table 5-5 and Table 5-6

Over both the short- and medium-term, repeatability was high, and the replication SD ranged from 1 to 3 dB. There was a slight improvement in repeatability with increasing frequency, and no consistent change in repeatability with stimulus level. The short-term repeatability was only slightly higher than the medium-term repeatability.

Table 5-5: Short-term replication SD of linear TEOAE level in dB (1/6-octave analysis)

Frequency	Click level (dB SPL)				
(kHz)	50	60	70	80	90
Broadband	1.0	1.72	1.91	2.16	2.0
1	2.15	1.39	2.21	3.15	3.02
2	1.02	2.41	1.64	1.92	2.10
3	1.05	0.97	0.81	1.69	1.35
4	1.78	1.51	1.04	1.71	1.71

Table 5-6: Medium-term replication SD of linear TEOAE level in dB (1/6-octave analysis)

Frequency	Click level (dB SPL)				
(kHz)	50	60	70	80	90
Broadband	1.68	1.99	2.11	2.54	2.58
1	2.72	1.79	2.30	3.42	3.65
2	1.04	2.71	1.39	2.40	2.36
3	0.97	1.55	1.11	1.76	1.81
4	1.34	1.56	1.27	2.12	2.34

5.5.3 MLS TEOAE

This section describes the normative properties of TEOAE obtained using maximum length sequences, and compares the effect of click rates of 50, 500 and 5000 clicks/s on MLS TEOAE.

5.5.3.1 Linear waveforms

The Natus instrument has a 17 ms time window and TEOAE waveforms from the Natus are plotted from 5 to 17.01 ms. In the same way as the ILO288, they consist of two waveforms A and B. Example waveforms from two different subjects at each click rate are shown in Figure 5-10. This showed a reduction in TEOAE level with increasing click rate, but the shape of the waveform was generally maintained, even at 5000 clicks/s.

The A and B waveform data were used to calculate the waveform repeatability within-session. This was calculated by measuring the cross-correlation coefficient of the A and B waveforms from 5 to 17 ms within each session. The mean correlation coefficients of the A and B waveforms are shown in **Figure 5-11**.

There was an increase in the correlation coefficient of the A and B waveforms with an increase in rate. This was most striking at the lowest intensity levels where the cross-correlation coefficient of the A and B waveforms at 5000 clicks/s was approximately one and a half times higher than that at 50 clicks/s.

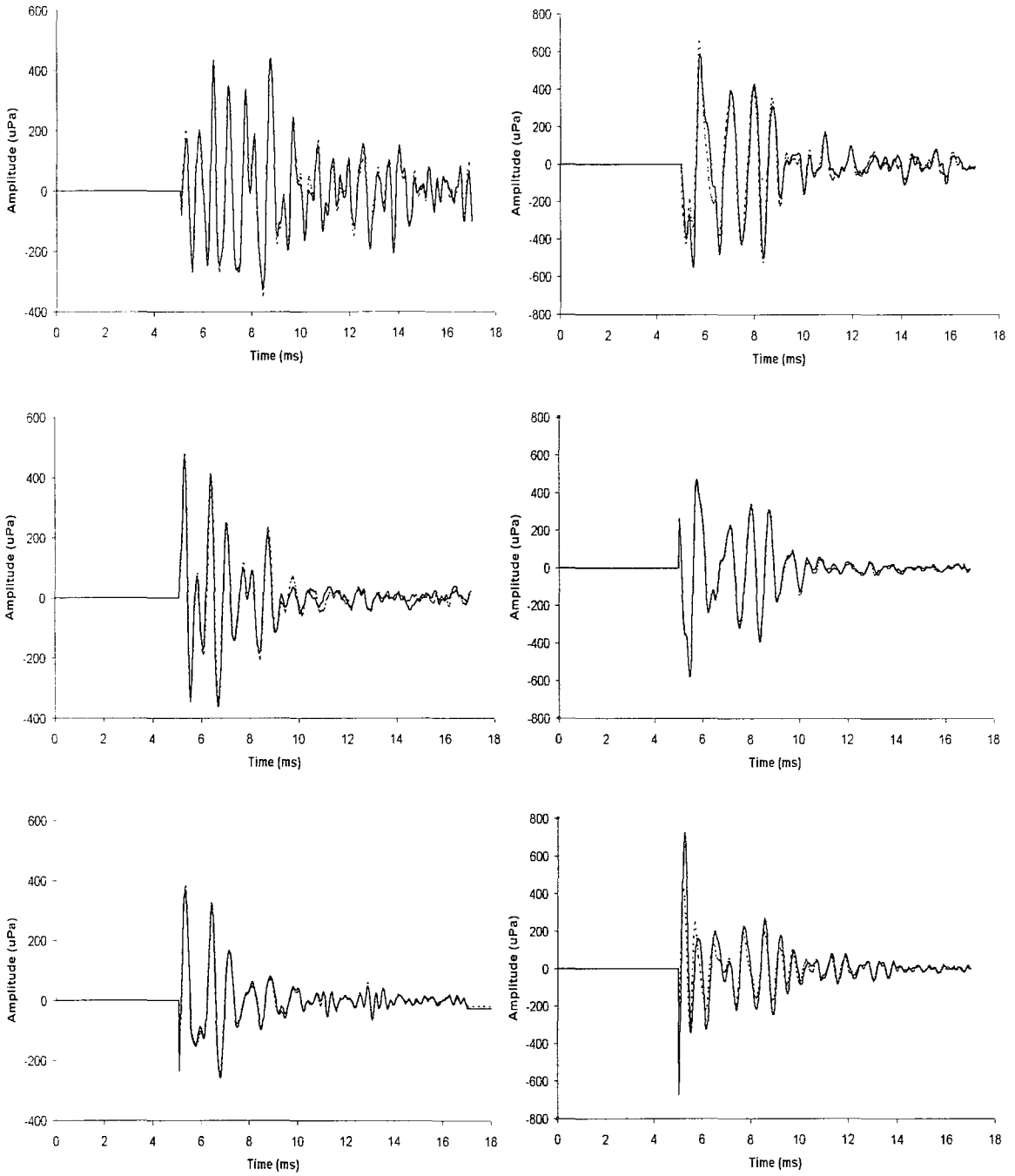


Figure 5-10: Example MLS TEOAE linear waveforms evoked by 80 dB click at different click rates. A) - C) Subject 5, click rates 50, 500 and 5000 clicks/s. D) - F) Subject 28, click rates 50, 500 and 5000 clicks/s. Key Wave A: solid line, wave B: dashed line.

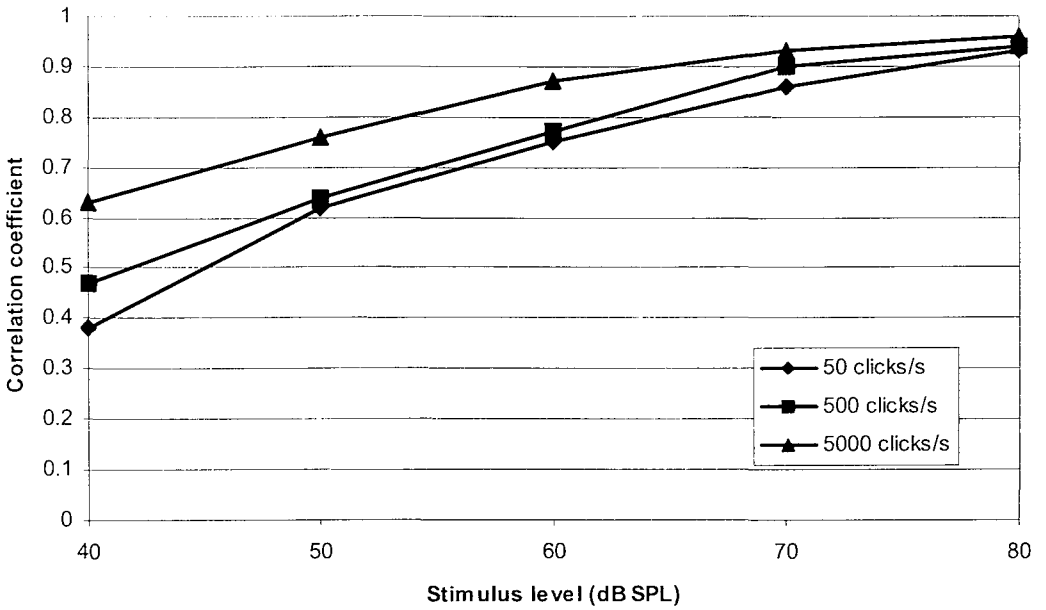


Figure 5-11: Mean cross-correlation coefficients of MLS TEOAE A and B waveforms calculated between 5-17 ms at three different click rates. The click rate of 5000 clicks/s has the highest repeatability at each stimulus level.

The between-session repeatability of the waveforms was assessed between sessions 3 and 4. Cross-correlation coefficients of the MLS TEOAE waveforms between session 3 and 4 were calculated. The mean group results are shown in Table 5-7.

This showed an increase in repeatability with increasing click level. Waveforms obtained at the higher click rates at the lower click levels were more repeatable than the conventional rate. At click levels of 70 and 80 dB, the repeatability was highest at a click rate of 50 clicks/s.

The effect of click rate on waveform morphology was assessed. Mean MLS TEOAE waveforms derived from sessions 3 and 4 were correlated across the different click rates. The mean group results are summarised in Table 5-8.

Table 5-8 showed that the correlation between the click rates increased with increasing click level. In general, the lowest correlation was between click rates of 50 and 5000 clicks/s. The highest correlation for all combinations was at 70 dB.

Table 5-7: Mean (SD) cross-correlation coefficients of the linear MLS TEOAE waveforms between sessions 3 and 4

Time (ms)	Rate (clicks/s)	Click level (dB SPL)				
		40	50	60	70	80
5.00-17.00	50	0.28	0.51	0.62	0.72	0.71
		(0.33)	(0.36)	(0.36)	(0.32)	(0.31)
	500	0.35	0.52	0.69	0.74	0.66
		(0.36)	(0.37)	(0.27)	(0.26)	(0.34)
	5000	0.47	0.60	0.71	0.71	0.60
		(0.40)	(0.36)	(0.31)	(0.31)	(0.41)
5.00-5.99	50	0.30	0.60	0.68	0.77	0.70
		(0.30)	(0.31)	(0.36)	(0.31)	(0.38)
	500	0.48	0.62	0.80	0.80	0.68
		(0.31)	(0.32)	(0.23)	(0.24)	(0.35)
	5000	0.44	0.60	0.76	0.71	0.58
		(0.43)	(0.37)	(0.29)	(0.30)	(0.49)
6.00-6.99	50	0.39	0.52	0.69	0.80	0.72
		(0.41)	(0.39)	(0.34)	(0.32)	(0.36)
	500	0.45	0.66	0.77	0.76	0.60
		(0.47)	(0.30)	(0.24)	(0.29)	(0.50)
	5000	0.57	0.71	0.80	0.74	0.53
		(0.41)	(0.36)	(0.31)	(0.42)	(0.59)
7.00-7.99	50	0.24	0.46	0.73	0.74	0.70
		(0.54)	(0.50)	(0.30)	(0.31)	(0.36)
	500	0.38	0.51	0.70	0.69	0.65
		(0.48)	(0.44)	(0.33)	(0.44)	(0.44)
	5000	0.56	0.66	0.77	0.74	0.57
		(0.38)	(0.36)	(0.32)	(0.39)	(0.60)
8.00-8.99	50	0.37	0.49	0.60	0.67	0.63
		(0.43)	(0.45)	(0.45)	(0.42)	(0.47)
	500	0.36	0.46	0.65	0.65	0.68
		(0.51)	(0.53)	(0.44)	(0.47)	(0.50)
	5000	0.46	0.62	0.68	0.74	0.62
		(0.57)	(0.51)	(0.46)	(0.41)	(0.54)
9.00-12.99	50	0.21	0.46	0.58	0.68	0.68
		(0.40)	(0.40)	(0.41)	(0.34)	(0.36)
	500	0.31	0.46	0.58	0.67	0.67
		(0.38)	(0.39)	(0.39)	(0.31)	(0.37)
	5000	0.41	0.50	0.63	0.72	0.73
		(0.45)	(0.46)	(0.44)	(0.31)	(0.28)
13.00-16.99	50	0.21	0.39	0.49	0.52	0.49
		(0.31)	(0.34)	(0.40)	(0.40)	(0.47)
	500	0.25	0.36	0.46	0.51	0.42
		(0.37)	(0.44)	(0.32)	(0.28)	(0.36)
	5000	0.24	0.46	0.51	0.59	0.65
		(0.45)	(0.38)	(0.37)	(0.38)	(0.28)

Table 5-8: Mean (SD) cross-correlation coefficients of linear waveforms at click rates of 50, 500 and 5000 clicks/s

Rate (clicks/s)	Click level (dB SPL)				
	40	50	60	70	80
50 v 500	0.42	0.61	0.70	0.76	0.77
	(0.27)	(0.24)	(0.17)	(0.12)	(0.14)
50 v 5000	0.42	0.59	0.67	0.67	0.63
	(0.26)	(0.23)	(0.18)	(0.19)	(0.19)
500 v 5000	0.53	0.65	0.72	0.71	0.70
	(0.29)	(0.26)	(0.14)	(0.17)	0.24)

5.5.3.2 Derived nonlinear waveforms

The nonlinear waveforms at stimulus levels of 60 and 70 dB were derived at click rates of 500 and 5000 clicks/s according to the methodology described previously. These click levels were chosen since the linear waveforms at these levels were the most repeatable. Figure 5-12 displays examples of linear and derived nonlinear waveforms from the same subject.

Repeatability of the derived nonlinear waveform within-sessions was not calculated, as only one waveform at each level was derived per session. However repeatability of the nonlinear derived waveform between sessions 3 and 4 was assessed. This was measured by calculating the cross-correlation coefficient of the nonlinear derived waveform over sessions 3 and 4, for each subject at 60 and 70 dB. The mean group results are shown in Table 5-9.

This showed an increase in the derived nonlinear waveform repeatability with an increase in click level. At the lower click level, repeatability was increased with an increase in click rate. There was no marked difference in repeatability between the click rates at 70 dB stimulus level. Compared to the linear waveform repeatability, the nonlinear repeatability was lower.

The effect of click rate on waveform morphology was assessed. Mean nonlinear waveforms derived from sessions 3 and 4 at each click rate were cross-correlated and the results are shown in Table 5-10.

The correlations between waveforms at different click rates were approximately the same for each click rate combination. The correlation between nonlinear waveforms at the different click rates was lower than the linear waveforms.

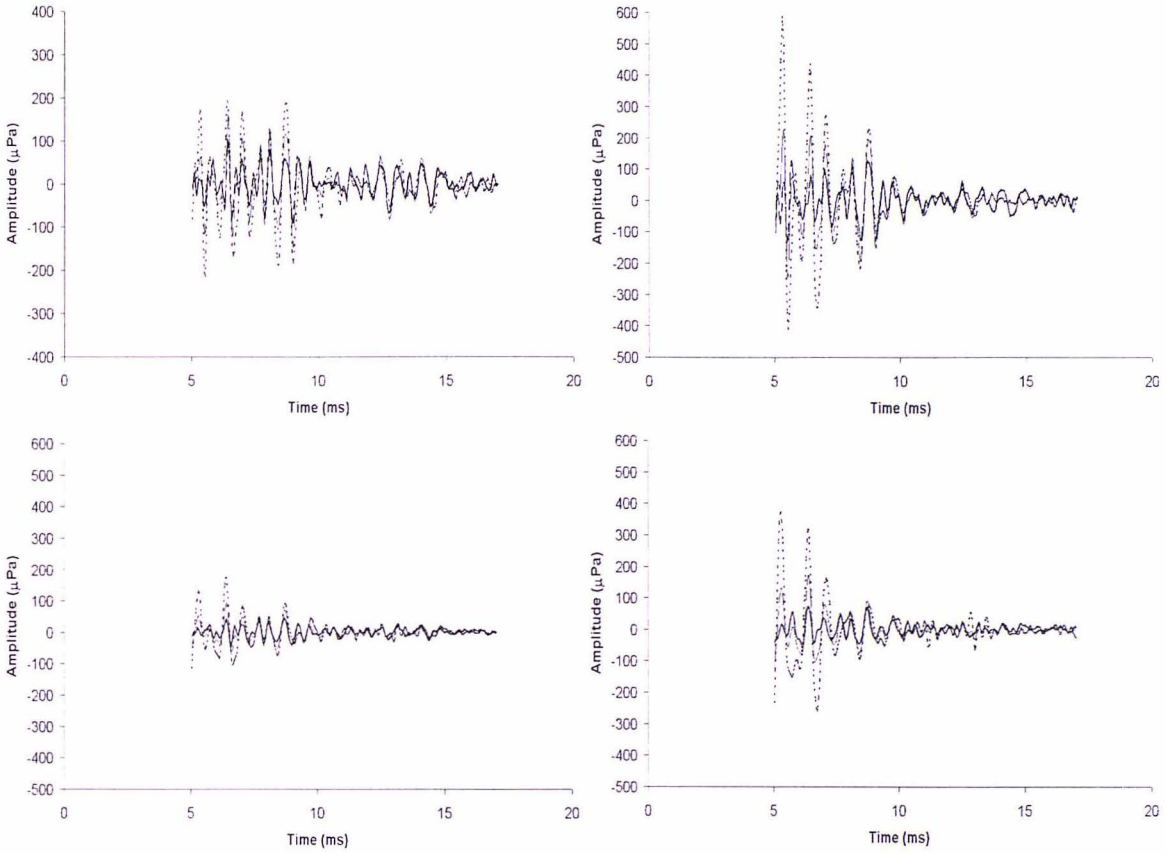


Figure 5-12 Example of derived nonlinear MLS TEOAE waveforms from subject 5 at A) 60 dB click level, 500 clicks/s, B) 70 dB click level 500 clicks/s, C) 60 dB click level, 5000 clicks/s and D) 70 dB click level, 5000 clicks/s. Nonlinear waveforms at 60 dB were derived from linear waveforms at 60 and 70 dB, and nonlinear waveforms at 70 dB were derived from linear waveforms at 70 and 80 dB. The derived nonlinear waveforms have similar level at both 60 and 70 dB stimulus level. Key: Dashed line: linear waveform at higher click intensity, thin solid line: linear waveform at lower click intensity, thick dark line: derived nonlinear waveform.

Table 5-9 Mean (SD) cross-correlation coefficients of the derived nonlinear MLS TEOAE waveforms between sessions 3 and 4

Time (ms)	Click rate (clicks/s)	Click level (dB SPL)	
		60	70
5.00-17.00	50	0.44	0.61
		(0.42)	(0.35)
	500	0.55	0.65
		(0.28)	(0.28)
	5000	0.60	0.63
		(0.30)	(0.35)
5.00-5.99	50	0.52	0.61
		(0.41)	(0.35)
	500	0.60	0.65
		(0.43)	(0.28)
	5000	0.68	0.63
		(0.31)	(0.35)
6.00-6.99	50	0.42	0.67
		(0.51)	(0.39)
	500	0.62	0.68
		(0.30)	(0.31)
	5000	0.74	0.74
		(0.26)	(0.37)
7.00-7.99	50	0.44	0.66
		(0.52)	(0.37)
	500	0.56	0.65
		(0.38)	(0.41)
	5000	0.59	0.75
		(0.34)	(0.35)
8.00 - 8.99	50	0.47	0.58
		(0.47)	(0.46)
	500	0.59	0.51
		(0.33)	(0.50)
	5000	0.58	0.64
		(0.45)	(0.50)
9.00 - 12.99	50	0.43	0.59
		(0.44)	(0.36)
	500	0.47	0.57
		(0.38)	(0.34)
	5000	0.56	0.65
		(0.43)	(0.37)
13.00 - 16.99	50	0.39	0.42
		(0.38)	(0.37)
	500	0.41	0.47
		(0.30)	(0.26)
	5000	0.41	0.54
		(0.34)	(0.34)

Table 5-10: Mean (SD) cross-correlation coefficients of derived nonlinear waveforms at click rates of 50, 500 and 5000 clicks/s

Click rate (clicks/s)	Click level (dB SPL)	
	60	70
50 v 500	0.52	0.59
	(0.25)	(0.22)
50 v 5000	0.53	0.53
	(0.25)	(0.23)
500 v 5000	0.54	0.54
	(0.26)	(0.18)

5.5.3.3 Frequency response

MLS TEOAE waveforms were assessed using FFT analysis. From here onwards, analysis was restricted to the linear waveforms due to their higher repeatability compared to the nonlinear responses. Figure 5-13 shows the mean frequency responses to clicks, at rates of 50, 500 and 5000 clicks/s at each stimulus level.

The mean frequency response of the MLS TEOAE recorded in the linear mode was similar at each click rate. The maximum level response at each rate occurred at approximately 1 kHz. One of the main differences between the responses at each click rate was the level of the noise floor. At 50 and 500 clicks/s it was approximately –20 to –25 dB, whereas at 5000 clicks/s it was approximately –30 to –35 dB. The other main difference was the maximum recordable frequency, which was approximately 4 kHz at 50 and 500 clicks/s, but increased to 5 kHz at 5000 clicks/s. Increasing click rate gave an increased signal-to-noise ratio, which was particularly marked at the lower intensity click levels.

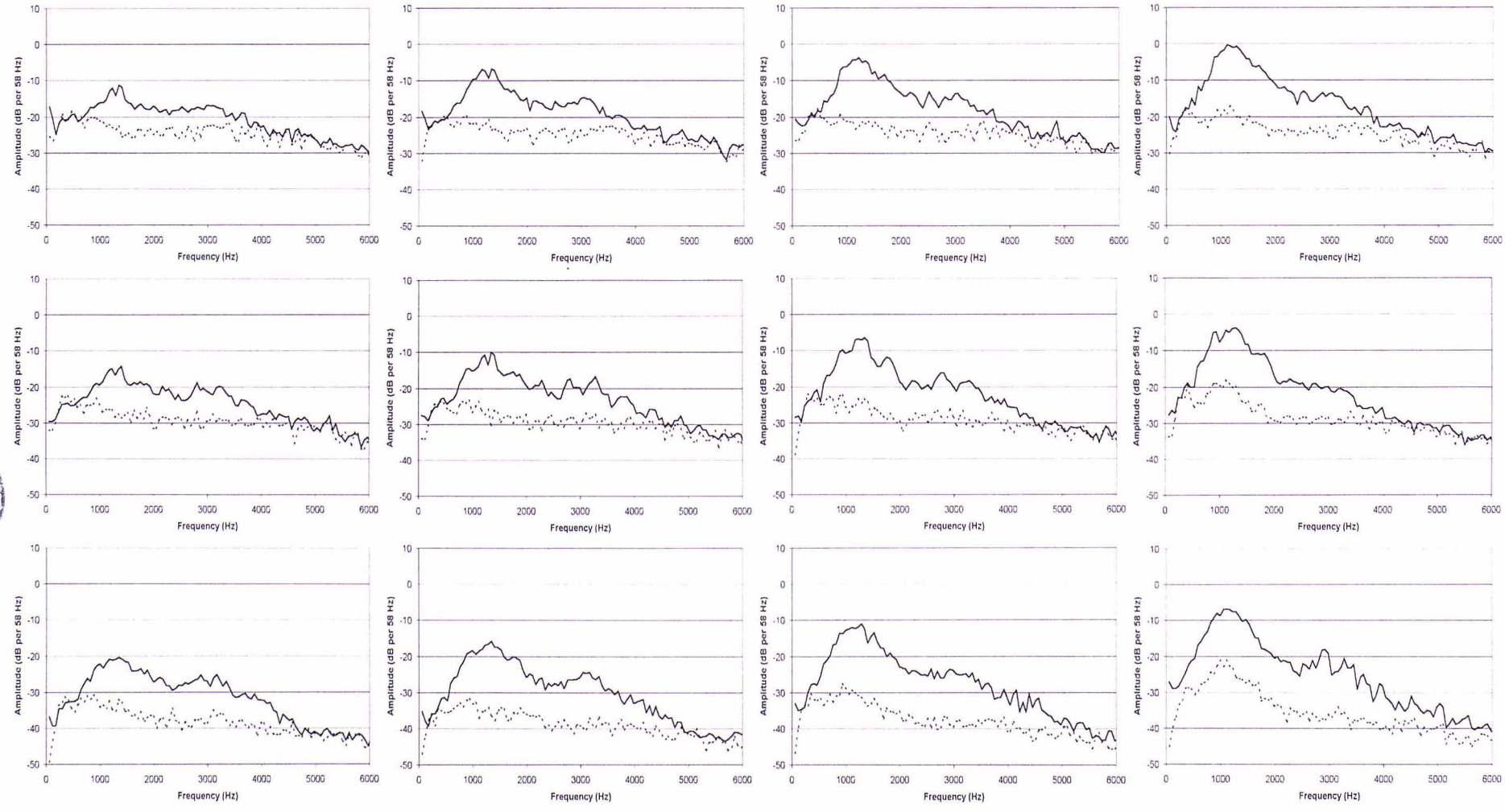


Figure 5-13: Mean MLS TEOAE FFT derived from linear waveforms (solid line) and mean noise floor level (dashed line) measured from normal hearing subjects at each level and click rate.

5.5.3.4 I/O functions

The level of MLS TEOAE waveforms in individual frequency bands was calculated and I/O functions were plotted at each frequency and click rate. Noisy data were excluded from analyses.

Individual subject I/O functions at each click rate are shown in Figure 5-14. The mean results are plotted in Figure 5-15 and Figure 5-16. These showed an increase in compression with increasing frequency, and I/O functions at 4 kHz were highly compressive. Comparing the I/O functions at different click rates showed functions with similar morphology at each frequency.

The slopes of the I/O functions were estimated using linear regression analysis using level data obtained between click levels of 50 to 80 dB. Table 5-11 summarises the median slope values of functions at each frequency and rate. This showed a decrease in slope value with increasing frequency consistent with Figure 5-14.

Table 5-11: Median and percentile slope values of linear MLS TEOAE I/O functions (dB/dB)

Clicks/s	Percentile	Frequency (kHz)				
		1	2	3	4	BB
50	25th	0.24	0.14	0.03	0.02	0.18
	Median	0.48	0.21	0.07	0.04	0.28
	75th	0.59	0.31	0.14	0.08	0.34
500	25th	0.32	0.08	–0.07	–0.07	0.16
	Median	0.41	0.15	0.04	–0.02	0.24
	75th	0.54	0.26	0.11	0.04	0.33
5000	25th	0.34	0.04	0.09	0.05	0.23
	Median	0.50	0.13	0.19	0.10	0.31
	75th	0.60	0.29	0.25	0.19	0.42

Key: BB broadband

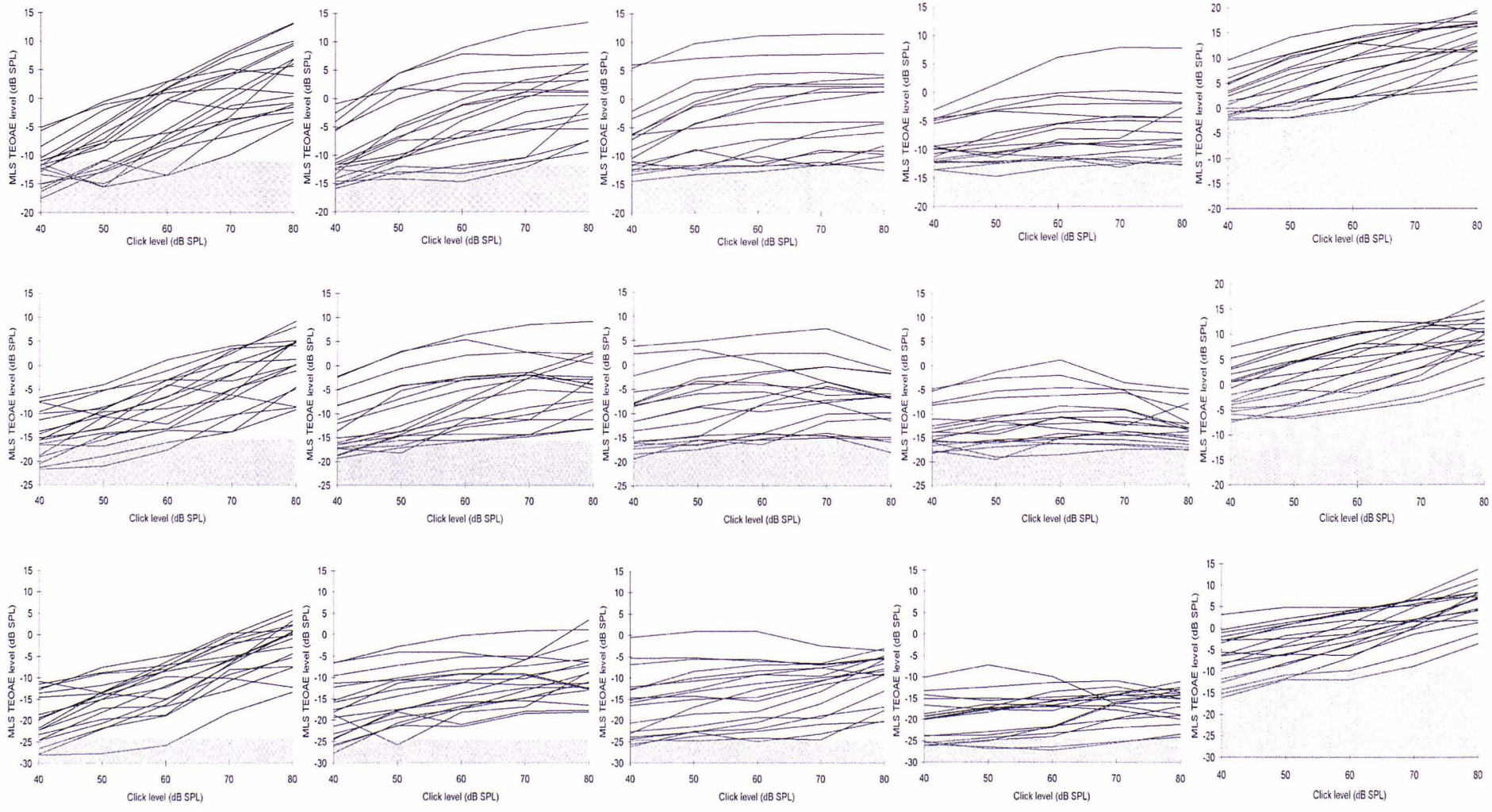
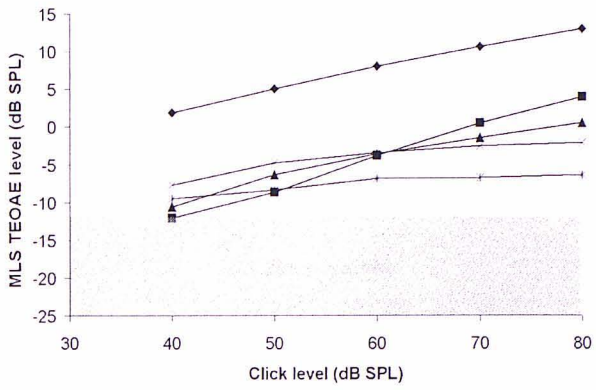
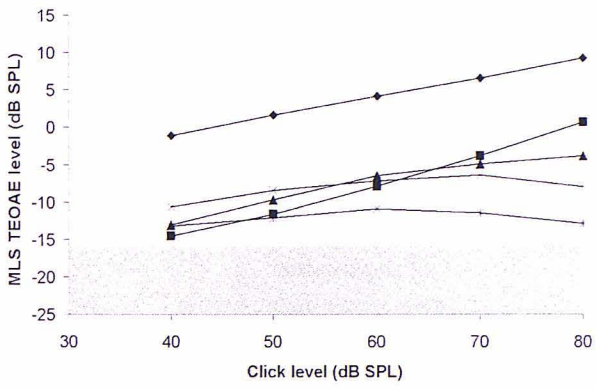


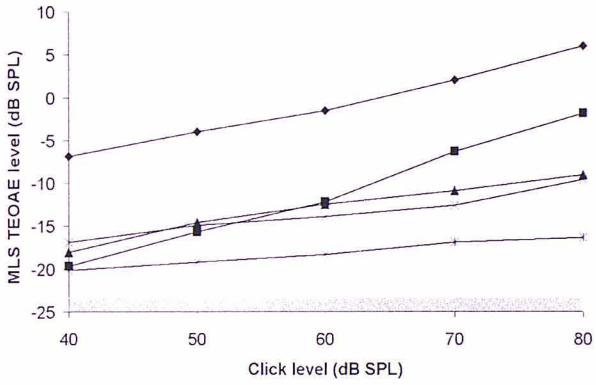
Figure 5-14: MLS TEOAE I/O functions across the frequency and click range, recorded from normally hearing subjects in the linear mode. Key to abbreviations – BB: broadband. Mean noise floor shown by shaded area.



A) 50 clicks/s



B) 500 clicks/s



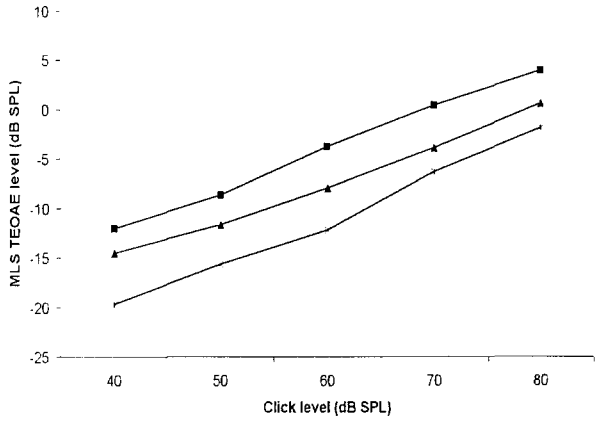
C) 5000 clicks/s

Figure 5-15: Mean MLS TEOAE I/O functions plotted by frequency at each click rate. A) 50 clicks/s. B) 500 clicks/s. C) 5000 clicks/s. Key - diamonds: BB, squares: 1 kHz, triangles: 2 kHz, crosses: 3 kHz, stars: 4 kHz. Mean noise floor shown by shaded area.

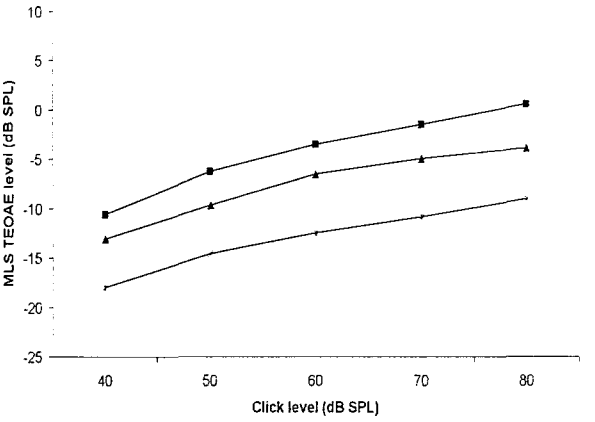
5.5.3.5 MLS rate suppression

MLS TEOAE rate suppression was assessed in the group of normal hearing subjects. Rate suppression was calculated as the difference in level (dB) between MLS TEOAE obtained at click rates of 50 and 500 clicks/s and 50 and 5000 clicks/s, denoted S_{500} and S_{5000} respectively. The rate effect was analysed across the frequency range and at each click intensity level. Frequency analysis of waveforms was performed in 1/6-octave bands.

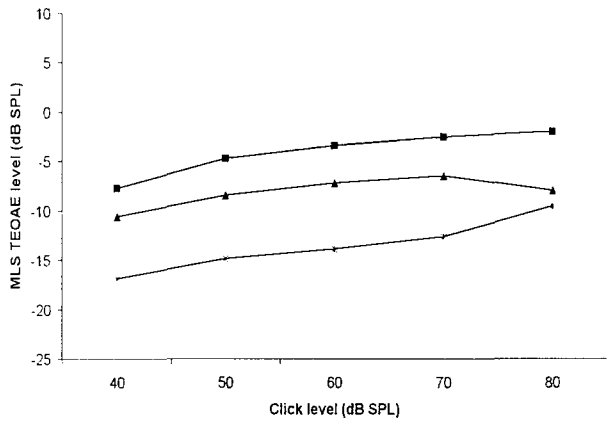
Figure 5-17 shows the mean S_{500} and S_{5000} values across frequency and level in the group of normal hearing subjects.



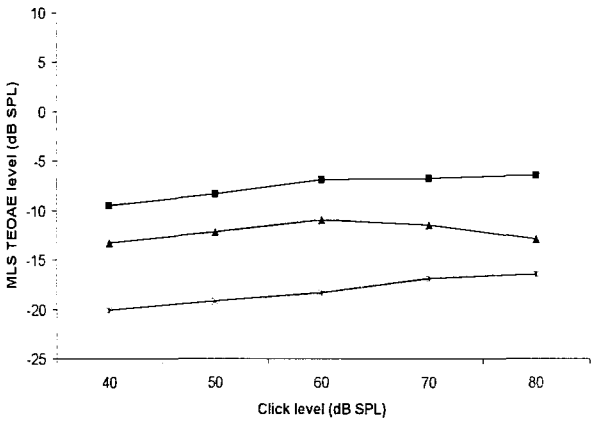
A) 1 kHz



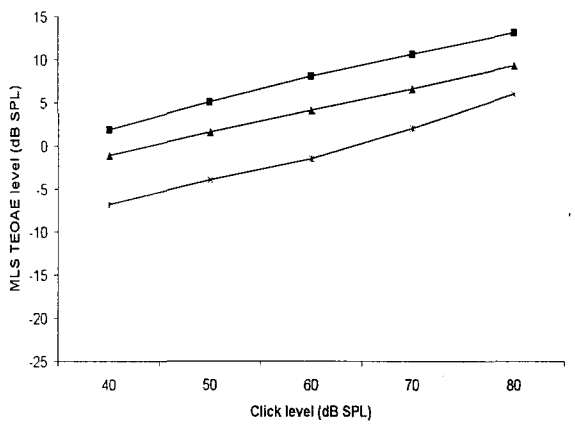
B) 2 kHz



C) 3 kHz



D) 4 kHz



E) Broadband response

Figure 5-16: Mean MLS TEOAE I/O functions plotted by click rate at each frequency. A) 1 kHz B) 2 kHz. C) 3 kHz. D) 4 kHz. E) Broadband.

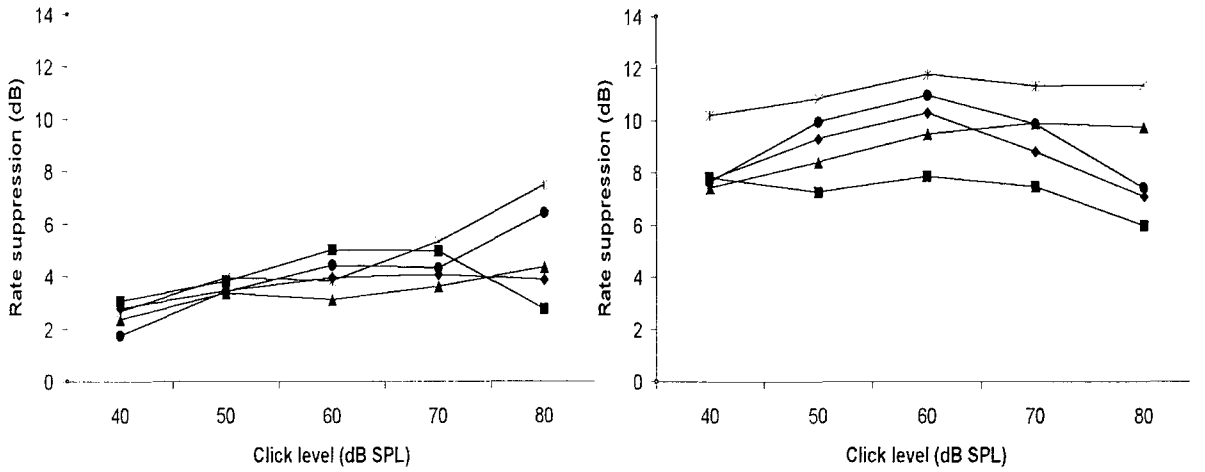


Figure 5-17: Mean MLS TEOAE rate suppression from normally hearing subjects. A) S_{500} B) S_{5000}. Key - squares: 1 kHz, triangles: 2 kHz, circles: 3 kHz, crosses: 4 kHz, diamonds: broadband response.

A repeated measures ANOVA was used to estimate the effect of frequency and level (within-subject factors) on MLS TEOAE rate suppression at 500 clicks/s. There was no significant overall effect of frequency on rate suppression. However there was a significant effect of click level at 3 and 4 kHz, and for the broadband responses, where rate suppression at 60, 70 and 80 dB was significantly higher than the responses at 40 dB.

A repeated measures ANOVA was used to estimate the effect of frequency and level (within-subject factors) on MLS TEOAE rate suppression at 5000 clicks/s. There was a significant overall effect of frequency on rate suppression, with rate suppression at 1 kHz significantly smaller than the results at 4 kHz ($P = 0.038$). There was a significant effect of level, with rate suppression at 40 dB significantly smaller than rate suppression at 60 dB ($P = 0.031$).

5.5.3.6 Test-retest repeatability

Test-retest repeatability of MLS TEOAE level was assessed over both the short- and medium-term using the same method described in section 5.5.2.5. Results are shown in Table 5-12 and Table 5-13.

For both the short- and medium-term, repeatability was high, and the replication SD ranged from 1 to 2 dB at 2-4 kHz and for the broadband response. Repeatability was lower at 1 kHz. There was a slight increase in repeatability with increasing frequency, and no consistent change in repeatability with stimulus level. Repeatability was slightly higher at higher click rates. The short- and medium-term repeatability values were similar.

Table 5-12: Short-term replication SD of linear MLS TEOAE level in dB (1/6 octave analysis)

Frequency (kHz)	Click rate (clicks/s)	Click level (dB SPL)				
		40	50	60	70	80
Broadband	50	1.32	1.06	1.34	1.07	1.49
	500	1.79	0.88	0.95	1.21	1.59
	5000	1.64	1.36	1.11	1.34	1.99
1	50	2.96	2.41	3.04	3.99	3.10
	500	4.39	3.90	2.73	3.52	4.71
	5000	4.21	3.02	3.93	2.34	3.59
2	50	2.33	2.06	1.86	1.62	2.42
	500	1.79	1.33	1.32	1.32	2.16
	5000	2.41	2.21	1.39	1.66	2.04
3	50	1.66	1.43	1.24	1.10	1.08
	500	1.71	1.20	1.33	1.05	1.17
	5000	1.16	0.95	0.68	1.13	1.35
4	50	1.83	1.52	1.47	1.13	1.85
	500	2.21	1.77	1.71	1.55	2.73
	5000	1.78	1.17	0.87	1.40	1.34

Table 5-13: Medium-term replication SD of linear MLS TEOAE level in dB (1/6 octave analysis)

Frequency (kHz)	Click rate (clicks/s)	Click level (dB SPL)				
		40	50	60	70	80
Broadband	50	0.97	0.92	1.01	0.84	1.00
	500	0.85	1.21	0.96	0.71	1.10
	5000	1.55	0.68	0.73	0.68	1.18
1	50	2.44	2.29	2.60	3.01	2.82
	500	2.74	2.58	2.62	2.47	2.54
	5000	2.55	1.76	1.63	1.14	1.53
2	50	1.74	1.39	1.22	1.42	1.49
	500	1.19	1.83	1.24	1.30	1.47
	5000	1.81	1.85	2.10	1.95	2.79
3	50	1.64	1.00	0.82	0.95	0.72
	500	1.08	0.82	1.08	1.21	0.63
	5000	1.25	1.33	1.05	1.05	0.91
4	50	0.90	1.08	1.08	1.25	2.02
	500	1.89	1.31	1.05	0.95	1.36
	5000	1.02	1.11	1.20	1.16	1.34

5.5.4 DPOAE

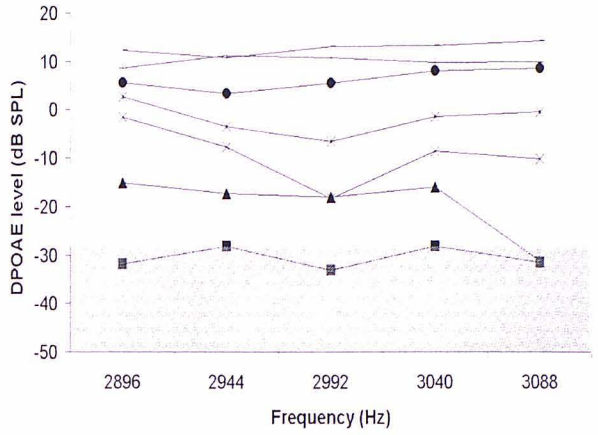
5.5.4.1 DP-grams

DP-grams are plots of DPOAE level against stimulus frequency, and are normally measured over a wide frequency range. When fine frequency spacing is used, fine structure is apparent within DP-grams, particularly at low intensity stimulus levels. For this reason, the level of a one-frequency DPOAE measure is dependent on its position along the DP-gram (i.e. within a peak or a trough). Fine structure is also affected by stimulus level, with an increasing frequency shift in fine structure as level increases. As stimulus increases, the one-frequency measure may be more likely to alter its position within a trough or near a peak.

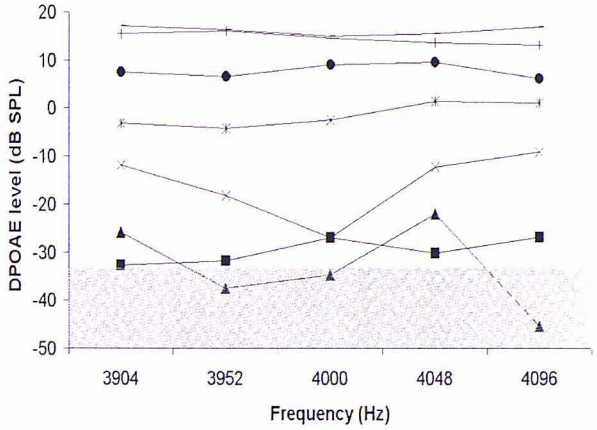
It was considered that repeatability of DPOAE level would be improved by averaging the level of several frequency points around the frequency of interest. This would remove errors caused by shifts in fine structure with stimulus level, and would be preferable to taking a single frequency measure of level. DP-grams recorded close to and around the frequency of interest are named here as mini DP-grams. Figure 5-18 gives examples of mini DP-grams recorded from one subject. The mini DP-grams at the lowest intensity levels were within the noise floor. However the results at 40 and 50 dB show wide variation in level even across the narrow frequency range of the mini DP-gram. At the highest intensity levels, the level values show little variation with frequency. Averaging across the frequency range, particularly at the middle intensity levels appeared to reduce the likelihood of recording in a trough along the DP-gram.

To assess whether averaging the mini DP-gram had a significant effect on DPOAE level, a paired t-test was used to compare the level obtained by averaging the mini DP-gram with the single frequency measure at 3, 4 and 6 kHz. This showed no significant group difference in DPOAE level between the two methods. The test-retest repeatability of the two methods was then compared by calculating the standard deviation on replication (see section 5.5.2.5 for method). This showed that the level obtained by averaging the mini DP-gram had a higher test-retest repeatability than the level obtained from a single frequency DPOAE. For this reason, all future DPOAE level measures were calculated by averaging the mini DP-gram. Frequency is specified as 3 kHz average, for example, to show that the value was calculated as the average of the 3 kHz mini DP-gram, rather than using a single frequency point at 3 kHz.

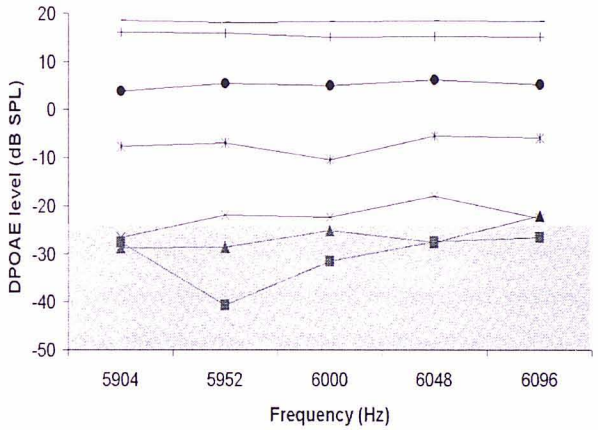
The effect of frequency on DPOAE was examined, see Figure 5-19. The mean DP-gram from all normal hearing subjects was plotted at each intensity level at each frequency. For each octave point, the average of the mini DP-gram was used. This showed a decrease in the average DPOAE level with increasing frequency. This effect was consistent across stimulus level.



A)



B)



C)

Figure 5-18: Example of the relationship between mini DP-grams and stimulus level (subject 19). A) 3 kHz. B) 4 kHz. C) 6 kHz. Key - f_2 stimulus level, squares: 10 dB, triangles: 20 dB, crosses: 30 dB, stars: 40 dB, circles: 50 dB, vertical bars: 60 dB, horizontal bars: 70 dB. Mean noise floor shown by shaded area.

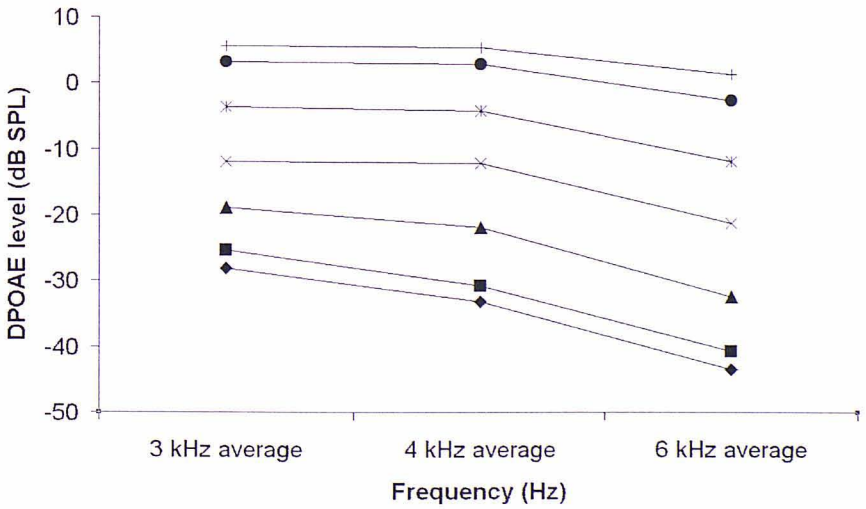


Figure 5-19: Mean normative DPOAE level across frequency. There is a reduction in DPOAE level with decreasing stimulus level and also with increasing frequency. Key - f_2 stimulus level, diamonds: 10 dB, squares: 20 dB, triangles: 30 dB, crosses: 40 dB, stars: 50 dB, circles: 60 dB, vertical bars: 70 dB.

5.5.4.2 Input-output functions

I/O functions were recorded from each subject using both the average and single frequency points at 3, 4 and 6 kHz. Figure 5-20 gives examples of the difference between I/O functions plotted using the average mini DP-gram and the I/O functions plotted from individual frequencies. This shows the wider variability in responses when single frequency points are used rather than averaging.

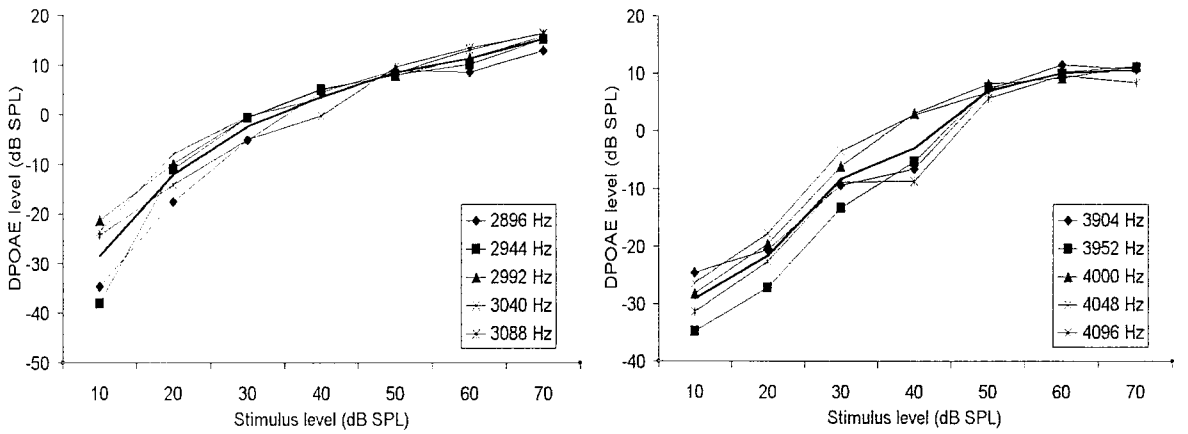


Figure 5-20: Individual DPOAE I/O functions recorded from subject 8. The solid lines are the I/O functions plotted at the different individual frequencies, showing the wide variation in level with frequency. The thick dark line shows the average DPOAE I/O function.

Individual subject DPOAE I/O functions (derived from the mean of sessions 3 and 4) are shown in Figure 5-21. This allows a comparison of I/O functions from both the average and single point frequency values. The average frequency values showed less variability between subjects, particularly at the lower stimulus levels.

Mean normative DPOAE I/O functions were plotted at each frequency, see Figure 5-22. Mean DPOAE I/O functions at 3 and 4 kHz were very similar. The I/O function at 6 kHz average was consistently lower in level across stimulus level.

Linear regression analysis was used to estimate the slope of each DPOAE I/O function between stimulus levels of 40-70 dB. These stimulus levels were chosen as they generated DPOAE levels above the noise floor in most subjects. Median and percentile values of the DPOAE slope values are shown in Table 5-14.

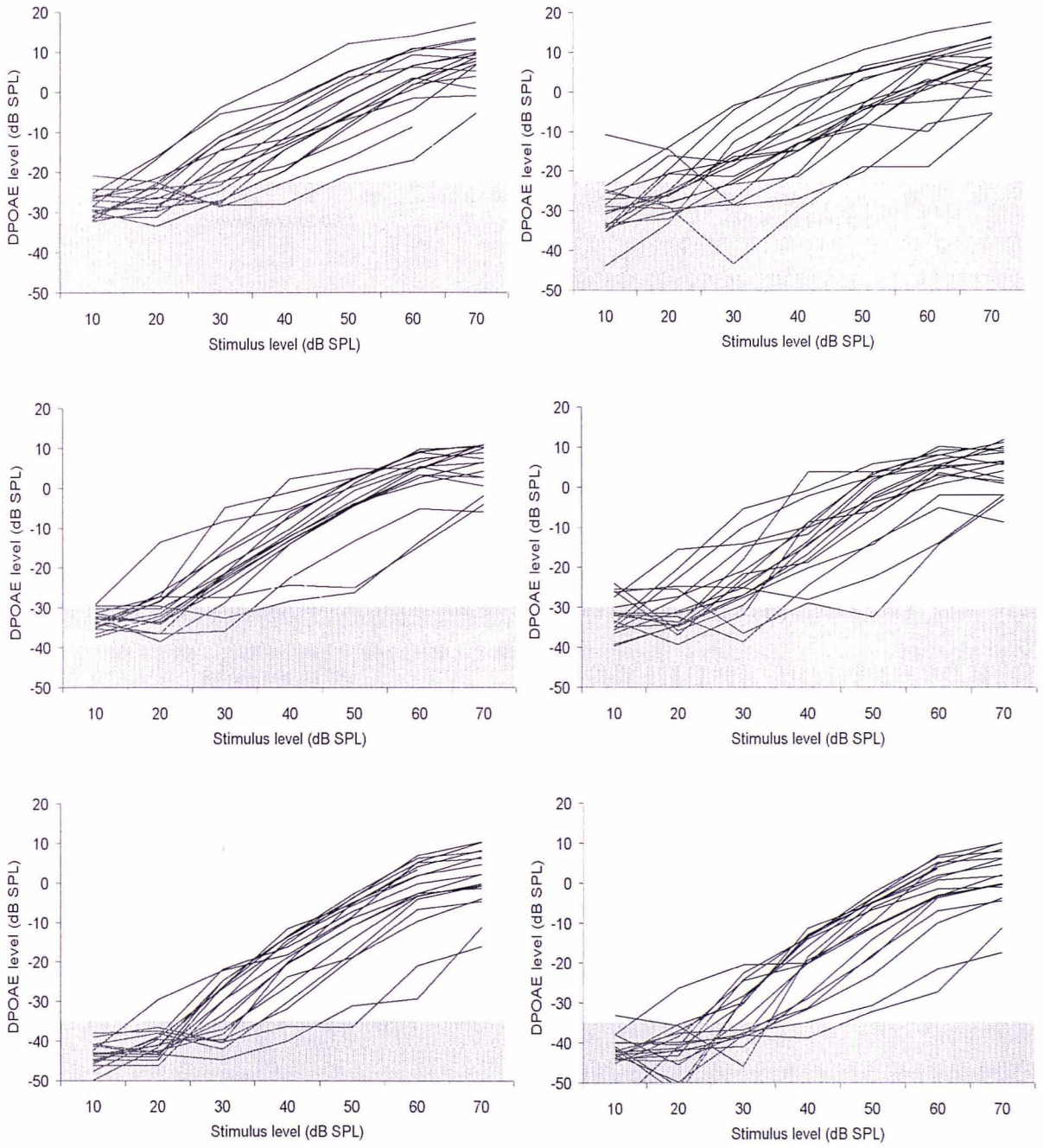
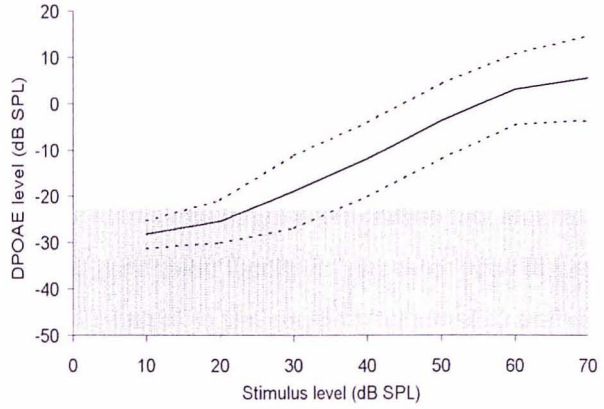
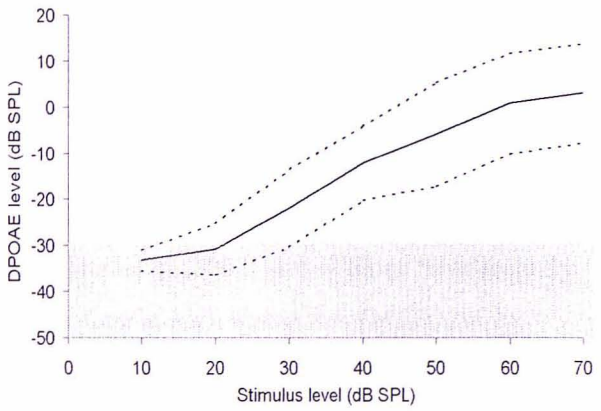


Figure 5-21: DPOAE I/O functions recorded from normal hearing subjects at A) 3 kHz average, B) 4 kHz average, C) 6 kHz average, D) 3 kHz, E) 4 kHz and F) 6 kHz. The I/O functions plotted from the single frequency point show more variability than the corresponding average frequency plots. Mean noise floor shown by shaded area.



A)



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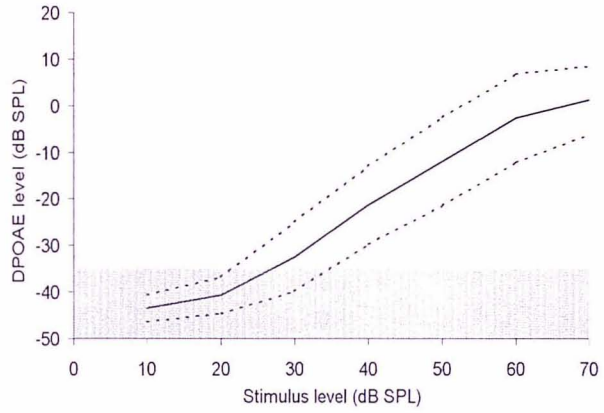


C)

Figure 5-22: Mean DPOAE I/O functions (solid line) ± one standard deviation (dashed line) at A) 3 kHz average, B) 4 kHz average and C) 6 kHz average. Mean noise floor shown by shaded area.

Table 5-14: Median and percentile slope values of DPOAE I/O functions (dB/dB)

Percentile	Frequency average (kHz)		
	3	4	6
25th percentile	0.49	0.49	0.69
Median	0.61	0.57	0.75
75th percentile	0.72	0.67	0.89

These results showed similar slope values of the DPOAE I/O functions at 3 and 4 kHz. There was an increase in slope value at 6 kHz, which is consistent with a decrease in compression with increasing frequency.

There are reports in the literature that DPOAE I/O functions from human ears contain non-monotonicities (e.g. Nelson and Kimberley, 1992). Non-monotonic growth is defined here as a reduction or saturation in DPOAE level followed by a further increase (>3 dB) in DPOAE level as stimulus increases. In animal ears, such non-monotonic growth was thought to be a result of the two-source active and passive generation mechanism, leading to phase cancellation. However the two-source generation theory has recently been disputed (Lukashin et al, 2002) and notches have recently been attributed to the cochlear response to a single primary tone at high intensities (Mills, 2002). Non-monotonic growth in human DPOAE I/O functions may also arise from this explanation, although they are less common than in small mammal ears.

DPOAE I/O functions from all subjects were examined for non-monotonic growth at frequencies of 3, 4 and 6 kHz and also 3, 4 and 6 kHz average. None of the I/O functions from the averaged mini DP-grams showed non-monotonic growth. At 3 kHz, only subject 30 showed consistent non-monotonic growth (using the definition above) in both session 3 and 4, see Figure 5-23.

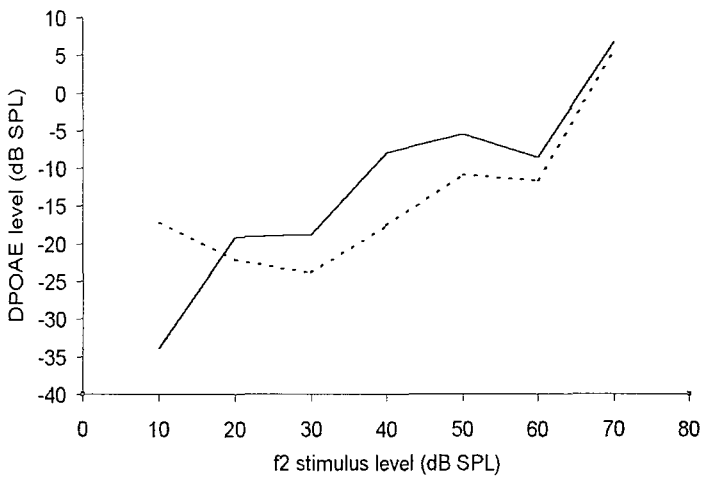


Figure 5-23: DPOAE I/O function recorded from subject 30 at 3 kHz showing clear non-monotonic growth around 50-60 dB. Non-monotonic growth is defined as a reduction or saturation in DPOAE level followed by a further increase in DPOAE level >3 dB as stimulus increases.

5.5.4.3 Test-retest repeatability

Test-retest repeatability of DPOAE measures was calculated (see section 5.5.2.5 for method). This was calculated using data from sessions 3 and 4. Table 5-15 and Table 5-16 summarise the short- and medium-term repeatability respectively for each DPOAE measure.

This showed an improvement in test-retest repeatability with increasing stimulus level. The poor repeatability at the low stimulus levels may be a result of recording results in the noise floor. There was no consistent effect of frequency on repeatability.

The repeatability of the average DPOAE results was higher than the repeatability of the single frequency DPOAE measure. This effect was particularly marked at the lower stimulus levels.

Table 5-15: Short-term replication SD of DPOAE level in dB

Frequency	f_2 stimulus level (dB SPL)						
(kHz)	10	20	30	40	50	60	70
3	6.53	7.10	4.14	3.80	2.44	2.41	4.25
3 average	3.58	3.70	2.59	3.14	2.73	1.77	3.04
4	8.46	5.45	4.14	3.23	2.25	1.30	1.50
4 average	3.95	2.34	2.62	1.75	1.74	1.16	1.34
6	6.59	7.40	4.13	3.69	3.34	3.35	3.56
6 average	2.67	3.03	3.87	2.25	2.73	3.11	3.52

Table 5-16: Medium-term replication SD of DPOAE level in dB

Frequency	f_2 stimulus level (dB SPL)						
(kHz)	10	20	30	40	50	60	70
3	4.66	4.32	5.76	4.19	3.69	4.06	4.21
3 average	2.17	2.60	3.28	2.69	3.15	2.91	4.89
4	3.92	4.22	3.28	3.49	3.47	3.25	3.55
4 average	2.14	2.05	2.73	3.33	3.55	3.16	3.28
6	4.82	5.89	4.51	4.51	4.41	4.66	5.04
6 average	2.74	2.77	3.13	3.11	4.21	4.72	4.96

5.5.5 Consequences of normative study

Of relevance for the cross-sectional analyses, linear TEOAE waveforms were shown to be similar to nonlinear TEOAE waveforms. Therefore linear TEOAE only are used for the cross-sectional analyses.

MLS TEOAE recorded at higher click rates had higher waveform repeatability at lower click levels than those recorded at the conventional rate. The relationship of MLS TEOAE responses at high click rates with HTL will be investigated and compared to responses obtained at conventional click rates.

DPOAE level calculated using the average of the mini DP-gram has a higher repeatability than DPOAE level recorded at a single frequency point. For this reason DPOAE results are analysed in terms of average mini DP-gram only for the cross-sectional study.

5.6 CROSS-SECTIONAL STUDY

This section describes the results of the cross-sectional study, comparing subjects with various degrees of sensorineural hearing loss. The relationship of each OAE measure to HTL was estimated by linear regression using OAE as the independent variable and HTL as the dependent variable.

5.6.1 Hearing threshold level

Forty-one subjects were tested; their individual HTL results are given in Appendix 5. Subjects were grouped at each frequency according to hearing threshold. Group 1 had HTL less than 0 dB, group 2: 0-9 dB, group 3: 10-19 dB, group 4: 20-29 dB, group 5: 30-39 dB and group 6: greater than 40 dB. Table 5-17 shows the number of subjects in each group for each frequency.

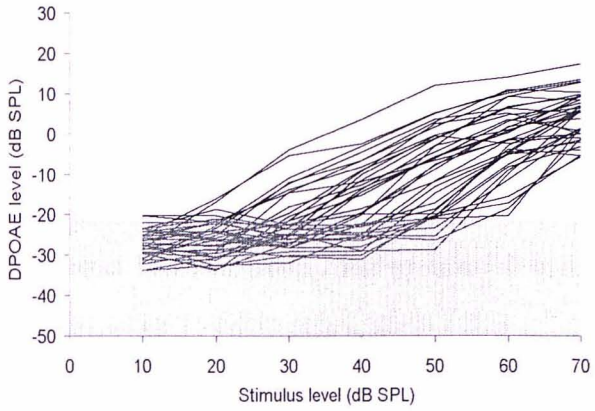
Table 5-17: Number of subjects in each HTL group at each frequency

HTL group	Frequency (kHz)			
	3	4	6	3-6 average
1 (<0 dB)	4	4	1	3
2 (0-9 dB)	15	10	1	10
3 (10-19 dB)	14	12	8	12
4 (20-29 dB)	6	7	9	8
5 (30-39 dB)	4	7	6	5
6 (>40 dB)	0	3	11	5

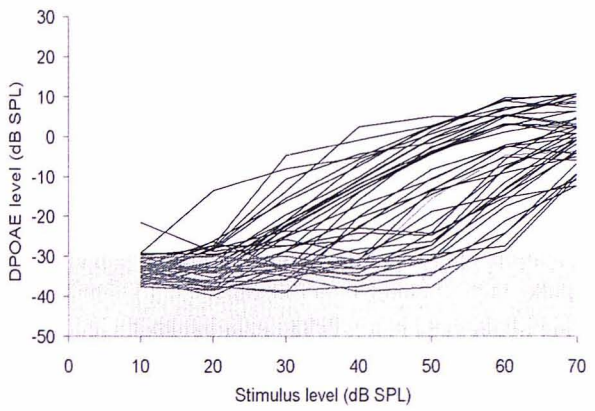
5.6.2 DPOAE

Results of the normative study showed that the average mini DP-gram DPOAE level had higher repeatability than single frequency DPOAE level. For this reason, all DPOAE level results are reported as average mini DP-grams.

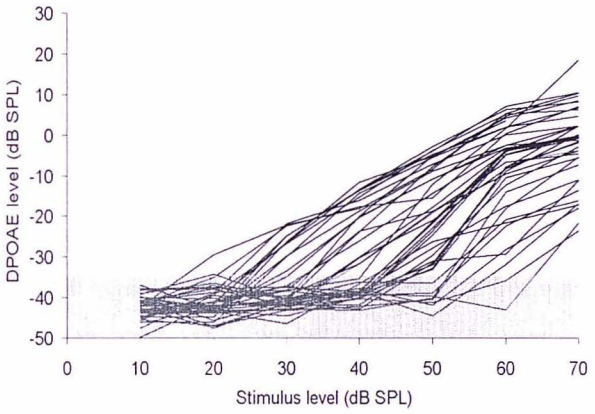
Mini DP-grams from each subject were averaged to give the mean DPOAE level for each stimulus level, at 3, 4 and 6 kHz. These values were used to plot I/O functions at each frequency, see Figure 5-24. All functions showed a general trend of increasing DPOAE level with increasing stimulus level. There was wide variation in the shape of the I/O functions, both between subjects and across frequency.



A)



B)



C)

Figure 5-24: Individual DPOAE I/O functions plotted across the frequency range. A) 3 kHz average. B) 4 kHz average. C) 6 kHz average. Mean noise floor shown by shaded area.

The variation in DPOAE I/O function morphology across HTL group was also examined. Mean DPOAE I/O functions were plotted according to HTL group, at each frequency. These are shown in Figure 5-25. For clarity standard deviation bars are not included.

It was hypothesised that differences in DPOAE I/O function between subjects with differing HTL would show differences in I/O function nonlinearity, and DPOAE level, according to the framework. Figure 5-25 shows that the results are consistent with this hypothesis. With increasing HTL group, there was a general trend for the I/O functions to shift across the right of the graph and to show a reduction in nonlinear compression. The largest differences in level between groups were at the mid-intensity stimulus levels, with larger DPOAE level associated with lower HTL. However, there was noticeable overlap in the I/O functions from different HTL groups. The degree of overlap depended on whether functions had been grouped according to a single frequency HTL or to the average of 3-6 kHz HTL frequencies.

I/O functions were analysed to assess for significant differences between groups. A repeated measures ANOVA was used. Frequency and level were specified as within-subject factors, and HTL group as

the between-subject factor. There was a significant effect of level and frequency on DPOAE I/O functions ($P<0.001$). There were significant group effects ($P<0.05$) at each frequency, dependent on the HTL grouping. When DPOAE I/O functions were grouped according to single frequency HTL, at 3 kHz there were significant differences between groups 1-2, and groups 3-6. At 4 kHz, there were significant differences between groups 1-3, and groups 4-6. At 6 kHz, there were significant differences between group 2 and groups 5-6, and between group 3 and group 6.

When DPOAE I/O functions were grouped according to the 3-6 kHz average HTL, at 3 kHz there was a significant difference between group 2 and 5. At 4 kHz, there were significant differences between groups 1-3 and groups 4-6. At 6 kHz, there were significant differences between group 1 and 5, between group 2 and groups 4-6, and between group 3 and group 5.

A gradation in DPOAE I/O function morphology was expected with increasing HTL group. However, there were only two main types of I/O function. One type of function, associated with low HTL was situated to the left of the graph, showed nonlinear compression and saturated at high stimulus levels. The functions associated with high HTL were more linear, showed little or no saturation at high intensity stimulus levels and were situated to the right of the graph. The HTL group cut-off between the two types of I/O function varied with frequency: at 3 kHz the cut-off was HTL greater than 10 dB, at 4 kHz greater than 20 dB, and at 6 kHz greater than 30 dB.

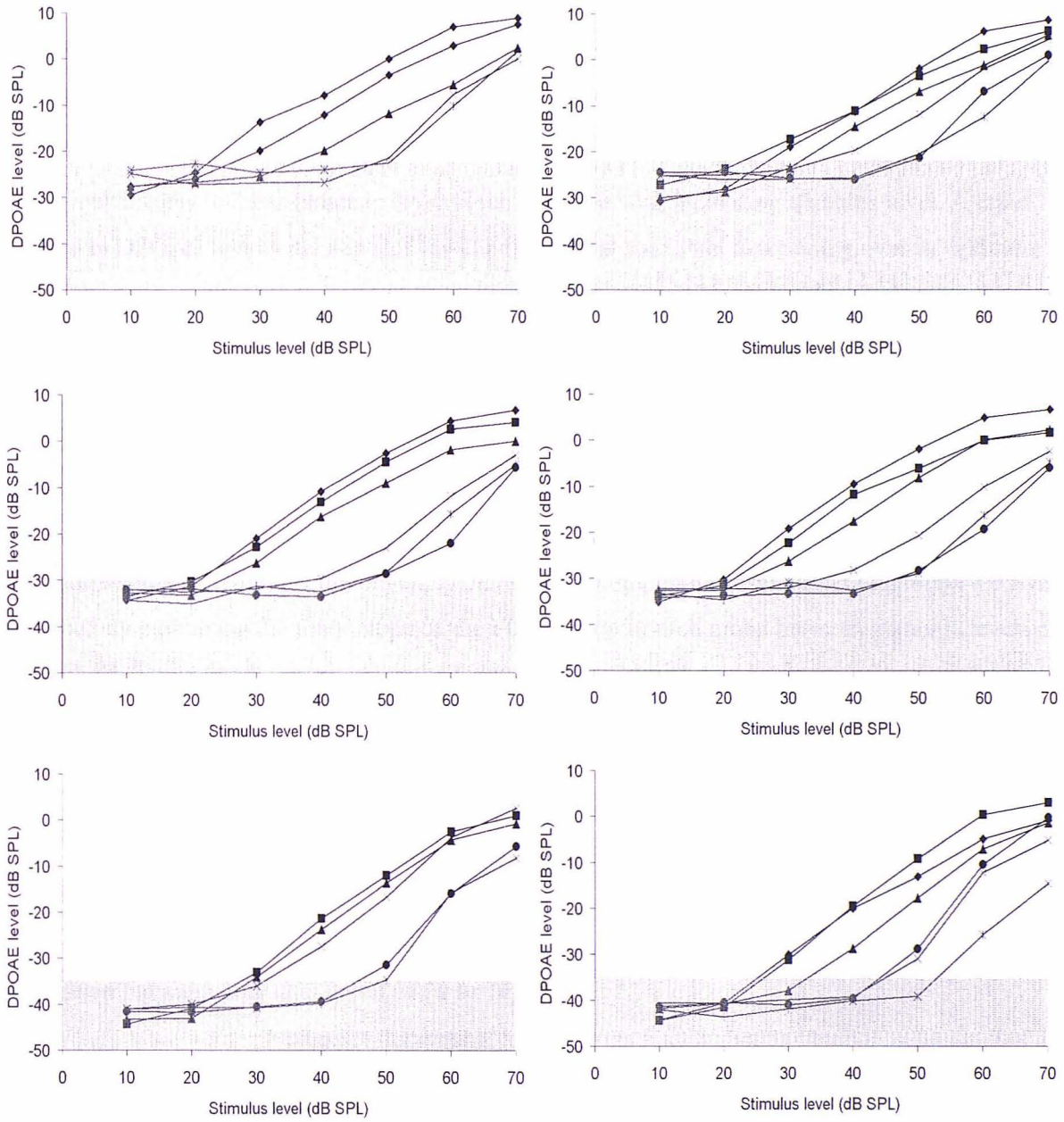


Figure 5-25: Mean DPOAE I/O functions plotted by HTL. Subjects were divided into HTL groups at the same frequency as f_2. A) HTL and DPOAE 3 kHz average. B) HTL and DPOAE 4 kHz average. C) HTL and DPOAE 6 kHz average. Subjects were also divided into HTL groups with HTL averaged across 3-6 kHz. D) HTL 3-6 kHz average and DPOAE 3 kHz average. E) HTL 3-6 kHz average and DPOAE 4 kHz average. F) HTL 3-6 kHz average and DPOAE 6 kHz average. Key: Group 1: diamonds, 2: squares, 3: triangles, 4: crosses, 5: stars, 6: circles. Note: At 6 kHz, group 1 was not included as it contained only 1 subject. Mean noise floor shown by shaded area.

Grouping functions according to the 3-6 kHz pure tone average resulted in a slightly different gradation. This grouping resulted in a clearer separation of DPOAE I/O functions between groups. The best separation between groups was at the mid-intensity stimulus levels. This suggests an influence of other hearing frequencies on the DPOAE.

OAE growth refers to the increase in level of the emission with increasing stimulus level. A rapid increase in DPOAE level with stimulus level indicates that the CA is functioning over its optimum range. The point at which there is little or no further increase in DPOAE level indicates that the CA is approaching or has reached saturation. Measuring DPOAE growth gives an indication of the stimulus range over which the CA is optimal. This can be quantified by calculating the slope of the growth function. A slope less than 3 dB/dB indicates compression in DPOAE growth and implies at least some CA saturation. The lower the slope, the greater the compression.

The framework predicts a reduction in compression with decreasing CA gain over mid-level stimuli, but no difference in growth at low levels. Differences in compression between HTL groups were first examined by measuring the mean slope of the I/O function in each group between stimulus levels of 50 to 70 dB. These levels were used as the functions showed evidence of compression over these stimulus intensities. They were also levels over which most subjects had measurable DPOAE. The mean slope data are shown in Table 5-18.

Table 5-18: Median slope value of the DPOAE I/O function (dB/dB) (linear regression performed between stimulus levels of 50 to 70 dB)

HTL group	Frequency (kHz)		
	3 average	4 average	6 average
1	0.41	0.51	
2	0.54	0.42	0.52
3	0.78	0.40	0.69
4	1.17	1.12	0.97
5	1.21	1.19	1.36
6		1.00	1.19

Examination of the DPOAE I/O functions showed a general reduction of compressive non-linearity with increasing HTL group. The lower slope values from the better hearing groups indicate a compressively nonlinear increase in DPOAE level with stimulus intensity, demonstrating compression of CA function over this stimulus range. The higher slope values from the worse hearing groups indicate a reduction in compression, consistent with a reduction in CA gain. However, no group approached the theoretical limit of 3 dB/dB for a non-compressive system.

Growth of DPOAE level was also examined at low DPOAE levels. The framework predicts a parallel right shift in DPOAE I/O functions as CA gain decreases. In the model, this shift occurs at the low DPOAE levels, with no change in the slope of the function. It was therefore predicted that there would be no difference in slope between the HTL groups at low DPOAE levels above the noise floor. The slope of the functions was calculated in each subject at DPOAE levels of –15, –20 and –30 dB SPL at 3, 4 and 6 kHz respectively. These levels were chosen as they were consistently above the noise floor in all subjects. The results are shown in Table 5-19.

Table 5-19: Median slope of the DPOAE I/O function at low intensity DPOAE levels (dB/dB)

HTL Group	Frequency (kHz)		
	3 average	4 average	6 average
1	0.89	1.10	
2	1.07	1.17	1.46
3	1.33	1.21	1.31
4	1.59	1.20	1.48
5	1.30	1.56	1.56
6		1.76	1.74

DPOAE growth at low OAE levels was approximately similar in each group, although there was a slight trend of increasing slope with increasing HTL group. This is consistent with the model indicating DPOAE growth out of the noise floor is similar in each HTL group. The model also predicts DPOAE growth at low level with a slope of 3 dB/dB, which was not observed here.

5.6.2.1 *DPOAE stimulus level* and HTL

The relationship between the stimulus level that evoked pre-set DPOAE level (denoted as *DPOAE stimulus level*) and HTL was assessed in subjects with differing HTL. *DPOAE stimulus levels* were estimated for each subject. These were the stimulus levels required to evoke DPOAE levels of –15, –15 and –30 dB at 3, 4 and 6 kHz respectively. These DPOAE levels were chosen, as they were the lowest values recordable in all subjects.

There was a wide range of values across subjects, spanning a range of approximately 50 dB across the frequency range. The relationship between *DPOAE stimulus level* and HTL was analysed using linear regression, the results of which are summarised in Table 5-20. This analysis showed highly significant relationships between *DPOAE stimulus level* and HTL at each frequency. The highest correlations were measured at 4 kHz. Examples of significant relationships are shown in Figure 5-26.

Table 5-20: Correlation coefficients relating *DPOAE stimulus level* (independent variable) and HTL (dependent variable)

DPOAE frequency (kHz)	HTL frequency (kHz)	Correlation coefficient (R)	Slope of regression line
3 average	3	0.72***	0.73
	4	0.64***	0.84
	6	0.50***	0.86
	3-6 average	0.64***	0.81
4 average	3	0.73***	0.65
	4	0.77***	0.93
	6	0.69***	1.04
	3-6 average	0.78***	0.88
6 average	3	0.60***	0.58
	4	0.73***	0.95
	6	0.64***	1.07
	3-6 average	0.71***	0.87

Key: * $P \leq 0.05$, ** $P \leq 0.01$ *** $P \leq 0.005$*

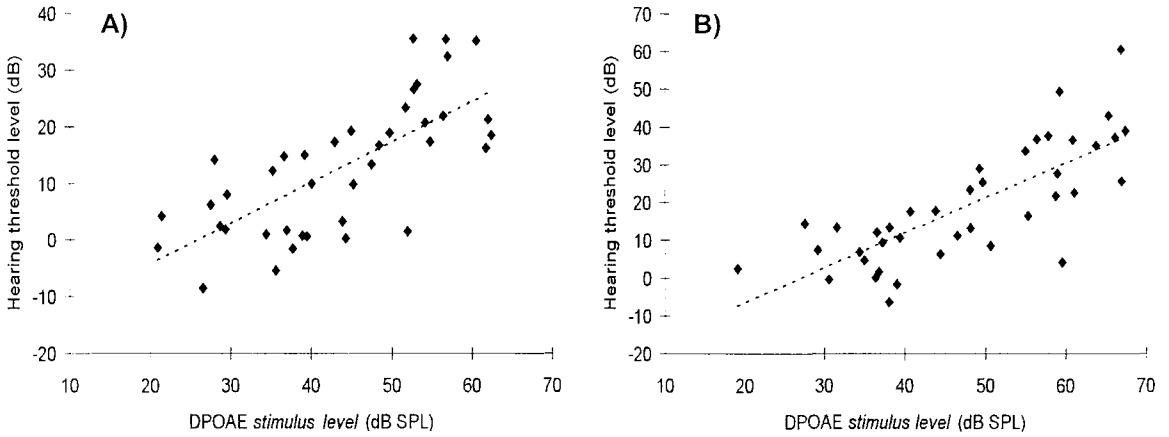


Figure 5-26: A) DPOAE stimulus level at a frequency of 3 kHz average, plotted against HTL at 3 kHz. B) DPOAE stimulus level at a frequency of 4 kHz average, plotted against HTL at 4 kHz. DPOAE stimulus level was the stimulus level required to evoke DPOAE level of −15 dB. Linear regression line plotted.

All graphs showed the same trend of increasing *DPOAE stimulus level* with increasing HTL. However there was wide variation in *DPOAE stimulus level* between subjects with similar HTL. At all frequencies, the correlation between DPOAE and HTL was highly significant ($P \leq 0.005$). The slope of the regression line relating the two variables quantifies the best fit between the two variables. A slope of 1 indicates a 1:1 relationship between *DPOAE stimulus level* and HTL. At a DPOAE frequency of 3 kHz, the slope value was less than 1 for each HTL frequency. This implies that a 10 dB change in DPOAE stimulus level is associated with a change in HTL less than 10 dB. As the DPOAE frequency increased, the slope of the relationship with HTL increased.

The R-square value indicates the proportion of the variance in the dependent variable (HTL) that is explained by the independent variable (*DPOAE stimulus level*). This value was between 0.4-0.6 at each frequency. This indicates that at 3, 4 and 6 kHz approximately 40-60% of the variance in HTL can be explained in terms of the differences in *DPOAE stimulus level*.

The *DPOAE stimulus level* results were then compared to the results of DPOAE level.

5.6.2.2 DPOAE level and HTL

It was predicted that changes in DPOAE level would also be related to changes in HTL. The relationship between DPOAE level and HTL was assessed in subjects with differing HTL.

DPOAE level was measured for each subject at each stimulus level that evoked DPOAE levels above the noise floor. DPOAE level varied across subjects and spanned a range of approximately 40 dB at each frequency. The relationship between DPOAE level and HTL was analysed using correlation analysis. Those with significant correlations were further analysed using linear regression, the results of which are summarised in Table 5-21.

Table 5-21 shows highly significant relationships between DPOAE level and HTL at each frequency. The highest correlations were obtained with DPOAE recorded with f_2 levels between 40 and 60 dB. DPOAE and HTL at 4 kHz showed the best relationship.

Figure 5-27 gives examples of the significant relationship between DPOAE level and HTL. These showed a general trend of decreasing DPOAE level with increasing HTL. The linear regression slopes varied from approximately –0.6 to –1.5 dB/dB. The maximum R-square values were approximately 0.6, indicating that DPOAE level explained a maximum of 60% of the variation in HTL. These results are similar to the relationship between *DPOAE stimulus level* and HTL.

Table 5-21: Correlation coefficients relating DPOAE level (independent variable) and HTL (dependent variable)

DPOAE frequency (kHz)	HTL frequency (kHz)	f_2 level (dB SPL)				
		30	40	50	60	70
3 average	3	–0.49***	–0.68***	–0.72***	–0.60***	
		(–0.84)	(–0.91)	(–0.82)	(–0.78)	
	4	–0.34*	–0.58***	–0.64***	–0.54***	
		(–0.77)	(–1.03)	(–0.95)	(–0.94)	
	6		–0.52***	–0.55***	–0.40*	
			(–1.18)	(–1.07)	(–0.89)	
	3-6 average	–0.36*	–0.61***	–0.66***	–0.52***	
		(–0.80)	(–1.04)	(–0.94)	(–0.87)	
4 average	3	–0.51***	–0.70***	–0.73***	–0.73***	–0.60***
		(–0.78)	(–0.72)	(–0.65)	(–0.77)	(–1.00)
	4	–0.53***	–0.73***	–0.76***	–0.79***	–0.59***
		(–1.09)	(–1.02)	(–0.92)	(–1.12)	(–1.35)
	6	–0.49***	–0.70***	–0.71***	–0.73***	–0.53***
		(–1.28)	(–1.22)	(–1.08)	(–1.31)	(–1.51)
	3-6 average	–0.54***	–0.76***	–0.78***	–0.80***	–0.60***
		(–1.05)	(–0.99)	(–0.88)	(–1.06)	(–1.29)
6	3	–0.54***	–0.66***	–0.61***	–0.38*	
		(–0.88)	(–0.74)	(–0.55)	(–0.36)	
	4	–0.51***	–0.71***	–0.70***	–0.52***	–0.36*
		(–1.13)	(–1.05)	(–0.85)	(–0.65)	(–0.60)
	6	–0. 40**	–0.66***	–0.62***	–0.43**	
		(–1.15)	(–1.26)	(–0.98)	(–0.70)	
	306 average	–0.50***	–0.72***	–0.69***	–0.47***	–0.31*
		(–1.05)	(–1.01)	(–0.79)	(–0.57)	(–0.49)

Correlation coefficients are shown, with significance values. Linear regression slope values are shown in parentheses. Key: $P \leq 0.05$, ** $P \leq 0.01$ *** $P \leq 0.005$*

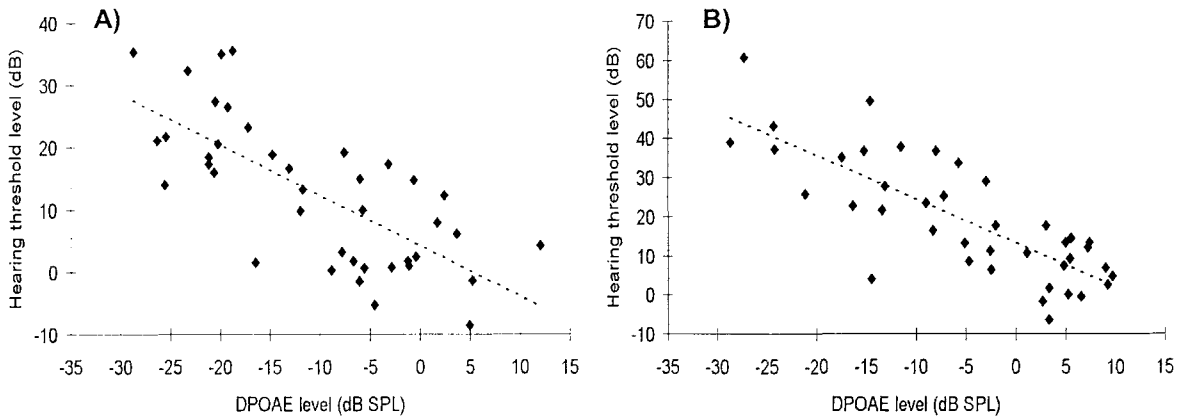


Figure 5-27: A) DPOAE level at a frequency of 3 kHz average plotted against HTL at 3 kHz. DPOAE level was evoked to f2 stimulus level of 50 dB. B) DPOAE level at a frequency of 4 kHz average plotted against HTL at 4 kHz. DPOAE level was evoked to f2 stimulus level of 60 dB. Linear regression line plotted.

5.6.3 TEOAE

This section discusses the results of TEOAE recorded using the ILO288 at the conventional rate. The TEOAE waveforms were analysed using the FFT analysis software of the ILO288, and verified by independent FFT analysis.

5.6.3.1 TEOAE I/O functions

The 1/6-octave band OAE level at each stimulus intensity was calculated. The values from sessions 3 and 4 were then averaged and used to plot I/O functions at each of these frequencies. These are shown in Figure 5-28.

The I/O functions showed a general trend of increasing TEOAE level with increasing stimulus level. There was wide variation in TEOAE level between subjects at each stimulus level. Most subjects had recordable TEOAE at 1 and 2 kHz, and also for the broadband response. However at the higher frequencies, many subjects only generated TEOAE at the maximum stimulus level of 90 dB. At 4 kHz, many subjects had no recordable TEOAE at any stimulus level. At frequencies of 3 and 4 kHz, the I/O functions were more compressive than at the lower frequencies.

The variation in I/O functions was examined with respect to HTL. The TEOAE results were divided into six groups according to the HTL of each subject, as shown in Table 5-17. Mean TEOAE I/O functions, plotted by HTL group at 3, 4 kHz and the broadband response are shown in Figure 5-29. For clarity, standard deviation bars are not included. TEOAE I/O functions at 1 and 2 kHz are not shown because HTL at 1 and 2 kHz was not recorded.

It was hypothesised that differences in TEOAE I/O functions with varying HTL would be similar in principle to the DPOAE model. Figure 5-29 show that the results at 3 and 4 kHz were not consistent

with this hypothesis. At 3 kHz, there was much overlap in responses and there were no consistent differences between groups. There was a general reduction in TEOAE level with increasing HTL, but no marked differences in linearity between groups. This was also observed at 4 kHz.

The results for the broadband responses were somewhat consistent with the predicted model of the framework. There was an increase in linearity with increasing HTL group. There was overlap between the normal hearing groups, but a clear separation between the normal hearing and the mild hearing loss groups.

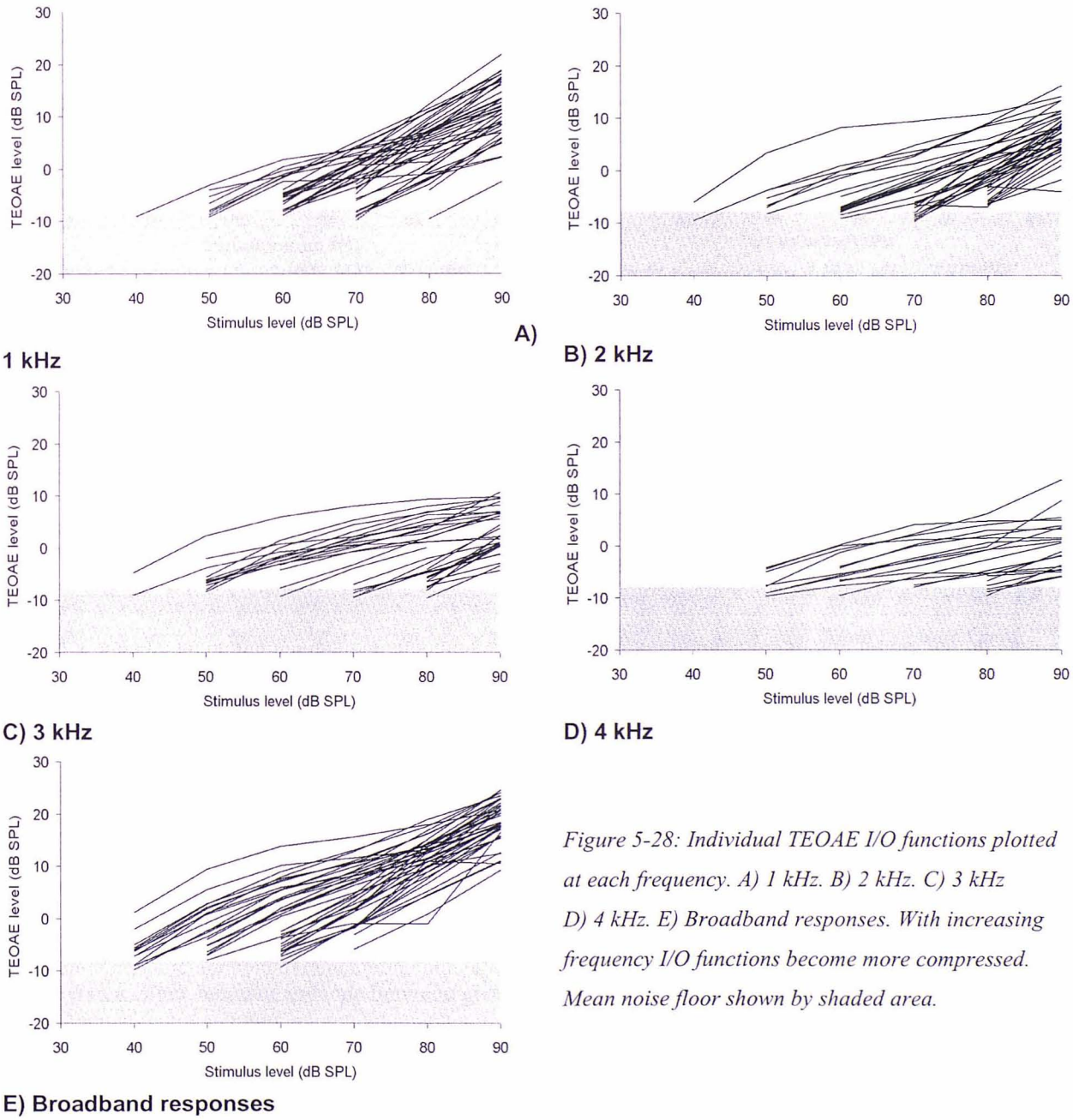
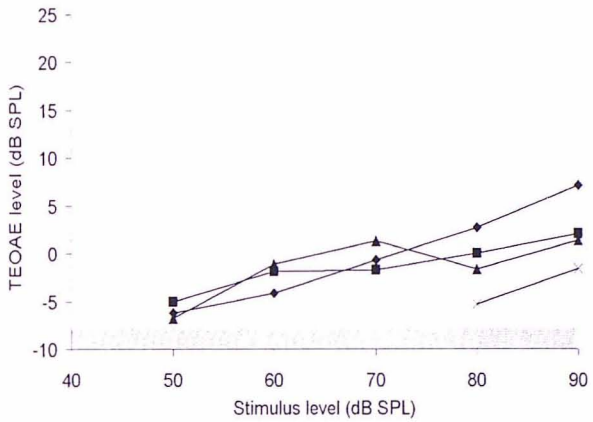
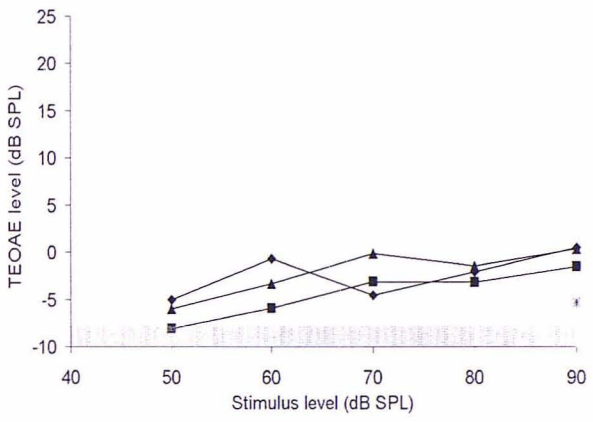


Figure 5-28: Individual TEOAE I/O functions plotted at each frequency. A) 1 kHz. B) 2 kHz. C) 3 kHz D) 4 kHz. E) Broadband responses. With increasing frequency I/O functions become more compressed. Mean noise floor shown by shaded area.

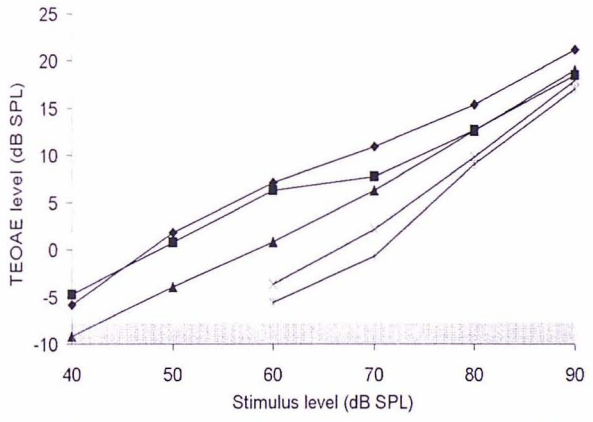
TEOAE I/O functions were analysed for significant differences between groups. A repeated measures ANOVA was used. Frequency and level were specified as within-group factors and HTL group as the between-subject factor. There was a significant effect of level and frequency on TEOAE I/O functions ($P<0.001$). There was no significant effect of HTL group on the I/O functions recorded at 3 or 4 kHz, and for the broadband responses.



A) Subjects grouped by 3 kHz HTL; TEOAE 3 kHz



B) Subjects grouped by 4 kHz HTL; TEOAE 4 kHz



C) Subjects grouped by 3-6 kHz average HTL; TEOAE BB

Figure 5-29: Mean TEOAE I/O functions plotted by HTL group. A) HTL 3 kHz and TEOAE 3 kHz. B) HTL 4 kHz and TEOAE 4 kHz. C) HTL 3-6 kHz average and broadband TEOAE responses. Key Group 1: diamonds, group 2: squares, group 3: triangles, group 4: crosses, group 5: stars, group 6: circles. BB: broadband. Mean noise floor shown by shaded area.

The I/O functions were examined for differences in TEOAE growth with HTL. The slope of each function was measured between stimulus levels of 60 and 90 dB, as most subjects had measurable TEOAE over these levels. The results are shown in Table 5-22

At 3 kHz, the growth of TEOAE level with increasing stimulus level varied between HTL groups. There was a slight increase in slope between group 2 and 3, indicating reduced compression. At 4 kHz, the slope value of the functions from groups 1-3 varied between 0.2-0.3 showing no difference in compression with increasing HTL. There were no data available for groups 4 and above, as these subjects did not generate TEOAE at stimulus levels below 90 dB. The results for the BB I/O functions

are in the expected direction, with a progressive approximately 0.1 dB/dB difference in slope for each 10 dB difference in HTL. Only the broadband TEOAE results were at all consistent.

Table 5-22: Median slope values of the TEOAE I/O function within each HTL group (dB/dB)

HTL Group	Frequency (kHz)		
	3	4	Broadband
1	0.33	0.24	0.41
2	0.33	0.40	0.56
3	0.43	0.30	0.55
4	0.85		0.80
5			0.79

5.6.3.2 TEOAE stimulus level and HTL

The relationship between *TEOAE stimulus level* and HTL was assessed in subjects with differing HTL. *TEOAE stimulus levels* were estimated for each subject. These were the stimulus levels required to evoke TEOAE levels of 0, –2 and –5 dB for BB, 3 and 4 kHz respectively. These values were chosen, as they were the lowest TEOAE levels recorded in all subjects.

There was a wide range of values across subjects, spanning a range of approximately 50 dB at 3 kHz, 46 dB at 4 kHz and 42 dB for the broadband responses. Unlike DPOAE, where it was possible to obtain a measure for all subjects, at 3 kHz measures were only obtained in 30 subjects, 20 subjects at 4 kHz and 42 subjects for the broadband responses. The relationship between *TEOAE stimulus level* and HTL was analysed using correlation analysis. The variables with significant correlations were then further analysed using linear regression analysis, the results of which are summarised in Table 5-23.

Table 5-23: Correlation coefficients relating TEOAE stimulus level (independent variable) and HTL (dependent variable)

TEOAE frequency (kHz)	HTL frequency (kHz)	Correlation coefficient (r)	Slope of linear regression line
3	3	0.40*	0.32
	4	0.55***	0.55
	3-6 average	0.45*	0.45
BB	3	0.58***	0.65
	4	0.59***	0.85
	3-6 average	0.59***	0.83

*Key: * P≤0.05, ** P≤0.01 *** P≤0.005*

Statistical analysis showed highly significant relationships between *TEOAE stimulus level* and HTL. Figure 5-30 gives an example of a significant relationship.

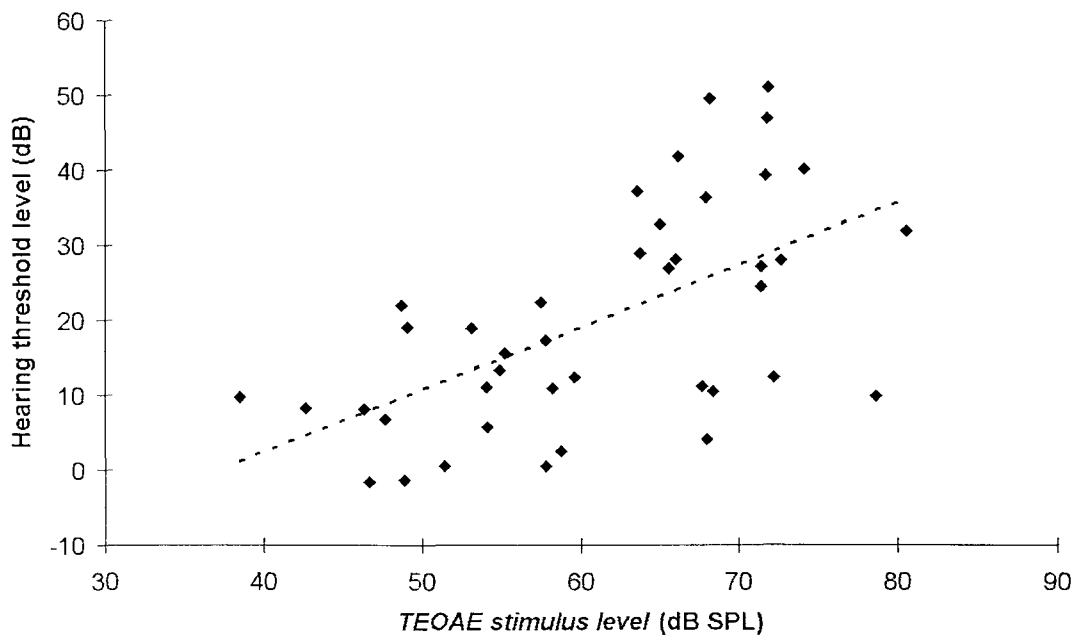


Figure 5-30: TEOAE stimulus level, broadband responses plotted against 3-6 kHz average HTL. TEOAE stimulus level was the stimulus level required to evoke TEOAE levels of 0 dB. Linear regression line plotted.

For the broadband responses, there was a general trend of increasing *TEOAE stimulus level* with increasing HTL, although there was wide variation between subjects. This was also observed at 3 kHz. At 4 kHz, no general trend was visible. Linear regression analysis showed significant relationships between HTL for 3, 4 and 3-6 kHz average and broadband and 3 kHz TEOAE responses. There were no significant associations for 4 kHz TEOAE and HTL.

There was wide variation among subjects in the relationship between TEOAE *stimulus level* and HTL, and this was reflected by the low R-square value showing that TEOAE explained only 20-30% of the variance in HTL. Some additional factor is required to explain HTL differences. At 4 kHz, the correlation between TEOAE stimulus level and HTL was not significant. This may be due to the lack of data at the upper end of the HTL scale.

The broadband *TEOAE stimulus level* responses had the highest correlation with HTL at all frequencies examined. However, even for the broadband responses, TEOAE explained only 35% of the variance.

5.6.3.3 TEOAE level and HTL

The relationship between TEOAE level and HTL was assessed in subjects with differing HTL.

TEOAE level was measured for each subject, at stimulus levels that evoked TEOAE above the noise floor. TEOAE level varied across subjects and spanned a range of approximately 20 dB at each frequency. The relationship between TEOAE level and HTL was analysed using correlation analysis, the results of which are summarised in Table 5-24.

Table 5-24 shows significant relationships between TEOAE level and HTL. There was a general trend of decreasing TEOAE level associated with increasing HTL, although there was wide variation between subjects. HTL in general showed the highest correlations with the BB TEOAE response. Figure 5-31 shows an example of a significant relationship.

The highest correlations were measured with stimulus levels of 60 and 70 dB SPL. This showed that TEOAE level explained approximately 30-40% of the variation in HTL. This was slightly higher than the relationship between *TEOAE stimulus level* and HTL. The linear regression slopes were in most cases greater than 1 dB/dB, showing that 10 dB differences in TEOAE level were associated with greater than 10 dB differences in HTL.

Table 5-24: Correlation coefficients relating TEOAE level (independent variable) and HTL (dependent variable)

TEOAE frequency (kHz)	HTL frequency (kHz)	Click level (dB SPL)					
		40	50	60	70	80	90
1	3			−0.50* (−1.42)	−0.49*** (−1.35)		
	4				−0.55*** (−1.92)		
	6			−0.48* −2.46	−0.58*** (−2.76)		
	3-6 average			−0.46* (−1.71)	−0.58*** (−2.03)		
2	3				−0.48** (−1.14)	−0.47*** (−1.23)	
	4				−0.50*** (−1.34)	−0.48*** (−1.55)	
	6				−0.43* (−1.60)	−0.38* (−1.70)	
	3-6 average				−0.48** (−1.32)	−0.45*** (−1.50)	
3	3					−0.38* (0.78)	−0.34* (−0.73)
	4				−0.54* (−1.32)	−0.52*** (−1.34)	−0.40* (−1.11)
	6						−0.33* (−1.24)
	3-6 average					−0.42* (−1.07)	−0.38* (−1.03)
4	3						
	4						−0.39* (−1.12)
	6						
	3-6 average						
BB	3			−0.55*** (−1.03)	−0.56*** (−1.23)	−0.39* (−1.04)	
	4			−0.67*** (−1.63)	−0.58*** (−1.64)	−0.36* (−1.21)	
	6			−0.63*** (−1.99)	−0.53*** (−2.02)		
	3-6 average			−0.66*** (−1.56)	−0.59*** (−1.64)	−0.34* (−1.16)	

Correlation coefficients are shown, with significance values. Linear regression slope values are shown in parentheses. Key: P≤0.05, ** P≤ 0.01 *** P≤0.005. BB: broadband*

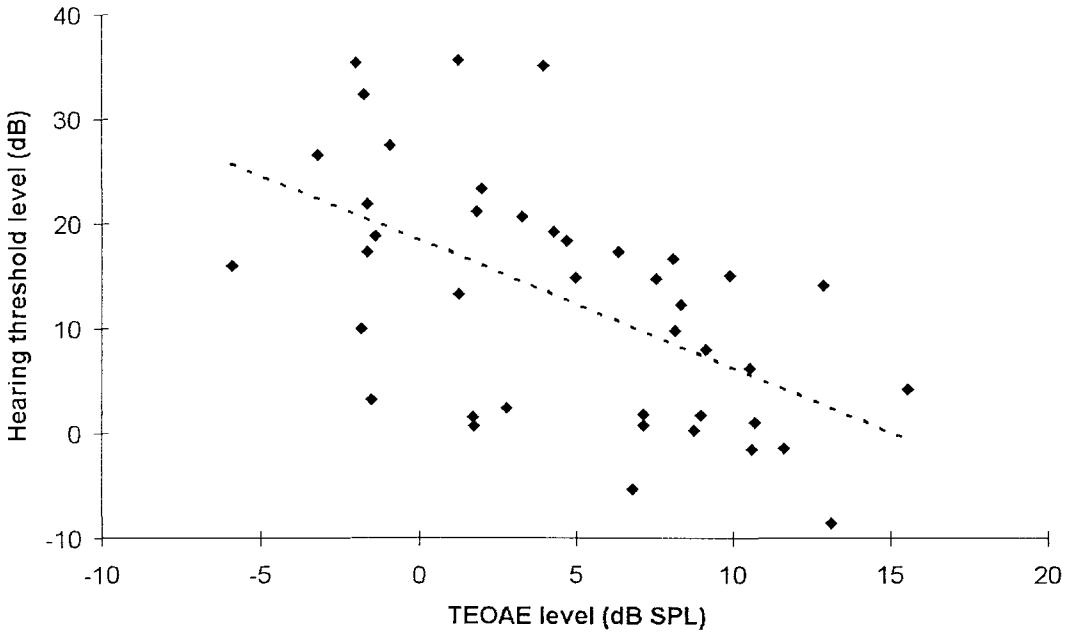


Figure 5-31: TEOAE level, broadband response, plotted against HTL at 3 kHz. TEOAE level was evoked to a stimulus level of 70 dB. Linear regression line plotted.

5.6.4 MLS TEOAE

This section compares the results from the MLS recording technique with those from the conventional method. It also discusses the results of MLS TEOAE recorded at click rates of 500 and 5000 clicks/s.

5.6.4.1 Comparison of MLS and conventional rate TEOAE

It was hypothesised that the use of maximum length sequences to record TEOAE would enable responses to be recorded at lower signal-to-noise ratios than with conventional TEOAE. It was considered that subjects with absent TEOAE on conventional recording might have detectable TEOAE responses with MLS recording. If this was the case the relationship between MLS TEOAE and HTL was expected to be better than that of conventional TEOAE and HTL.

The percentage of subjects with detectable MLS TEOAE recorded at 5000 clicks/s was compared with the percentage of those with detectable MLS TEOAE recorded at 50 clicks/s (equivalent to the conventional recording). All comparisons were made using recordings from on the Natus machine, as the aim was not to compare different equipment but to compare MLS versus conventional recording. A detectable response was defined as a TEOAE that was 3 dB or more above the noise floor. Figure 5-32 shows the percentage of subjects (both normal hearing and hearing impaired) with measurable TEOAE for the two recording methods.

This showed that MLS TEOAE were recordable in a higher percentage of subjects than conventional TEOAE. This was particularly striking at the lower intensity levels. At a stimulus level of 40 dB,

conventional TEOAE responses were detected in approximately 30% of subjects. In contrast, MLS TEOAE responses were detected in approximately 50% of all subjects. The percentage of subjects with conventional TEOAE responses increased with increasing click level. However even at a click level of 80 dB, high frequency TEOAE (3 and 4 kHz) were recorded in more subjects using the MLS technique than using the conventional method.

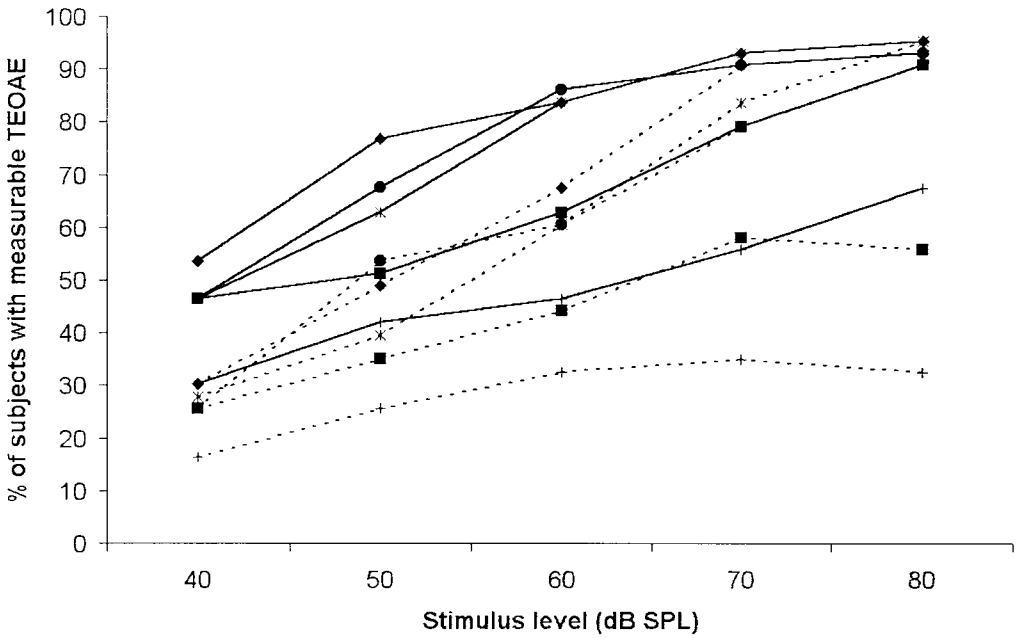
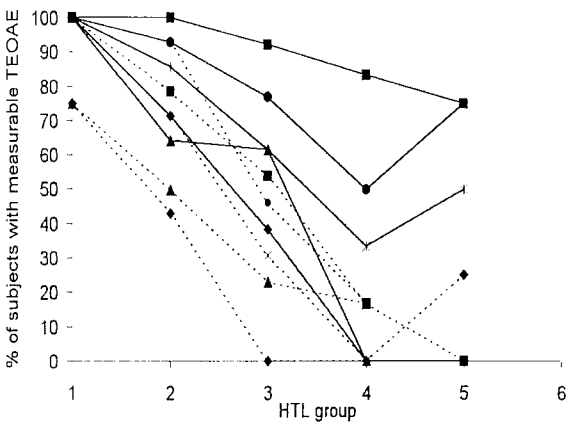
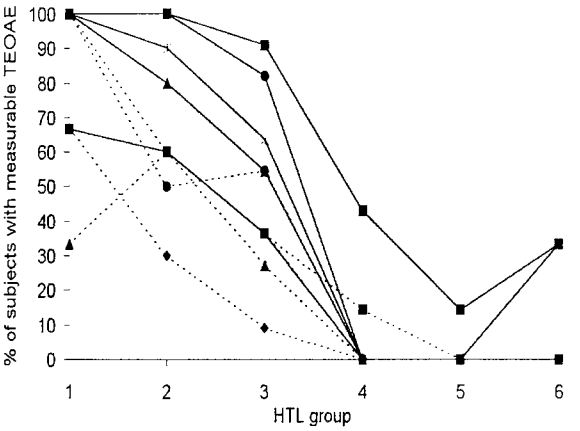


Figure 5-32: Comparison of percentage of subjects (normal hearing and hearing impaired) with recordable TEOAE using a click rate of 50 clicks/s (broken line) compared to a click rate of 5000 clicks/s (solid line) across the frequency range. Recordings were all made on the Natus machine. A greater percentage of subjects had recordable TEOAE at the lower intensity levels when recorded at a click rate of 5000 clicks/. Key to symbols – 1 kHz: diamonds, 2 kHz: circles, 3 kHz: squares, 4 kHz: pluses, broadband responses: stars.

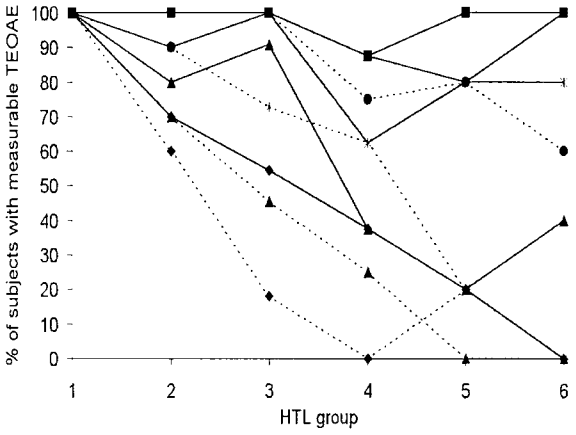
The data were examined to investigate whether there was any difference in the relationship between TEOAE and HTL for the different recording techniques. Figure 5-33 shows the percentage of detectable TEOAE plotted by HTL group. In general, MLS TEOAE were detectable in more subjects than the conventional TEOAE. This was particularly marked in the higher HTL groups and at the lower stimulus levels. This is likely to be a result of the lower noise floor of approximately –25 dB at 5000 click/s, compared to approximately –12 dB at 50 clicks/s.



A) 3 kHz



B) 4 kHz



C) Broadband

Figure 5-33: Comparison of the percentage of subjects (normal hearing and hearing impaired) with recordable TEOAE using a click rate of 50 clicks/s (broken line) compared to a click rate of 5000 clicks/s (solid line) at each HTL group. A) 3 kHz TEOAE and 3 kHz HTL. B) 4 kHz TEOAE and 4 kHz HTL. C) Broadband TEOAE responses and 3-6 kHz average HTL. Key to symbols: 40 dB: diamonds, 50 dB: triangles, 60 dB: stars, 70 dB: circles, 80 dB: squares.

5.6.4.2 MLS TEOAE I/O functions

Results at 50 clicks/s are not described as they duplicate those described in section 5.6.3.1, recorded using the ILO288. The 1/6-octave band OAE level at each stimulus intensity and click rate was calculated. The broadband response was also calculated. The values from sessions 3 and 4 were then averaged and used to plot I/O functions at each of those frequencies. See Figure 5-34.

At 500 clicks/s, the broadband response and those at 1 and 2 kHz showed a general trend of increasing TEOAE level with increasing stimulus level. At 3 and 4 kHz, there was little or no increase in level with increasing stimulus level, giving a highly compressed I/O function. The noise floor was approximately –16 dB SPL. At 5000 clicks/s, a similar pattern of I/O function was also observed, although the noise floor was lower at approximately –25 dB SPL.

The MLS TEOAE results were divided into five groups according to the HTL of each subject, as shown in Table 5-17. TEOAE I/O functions at 1 and 2 kHz are not shown because HTL at 1 and 2 kHz was not recorded. Figure 5-35 shows the mean MLS TEOAE I/O functions plotted by HTL groups. For clarity, standard deviations bars are not shown.

I/O functions were analysed for significant differences between groups. A repeated measures ANOVA was used. Frequency, level and rate were specified as within-subject factors, and HTL group as the between-subject factor. There was a significant effect of frequency, level and rate on MLS TEOAE I/O functions ($P<0.001$). There were significant group effects as expected, between I/O functions from the normally hearing groups (groups 1-3) and the hearing loss groups (groups 4-5) where the I/O functions were mainly within the noise floor.

At both 3 and 4 kHz, and at click rates of 500 and 5000 clicks/s, there was a general trend of a downward shift in I/O function with increasing HTL group. The results of groups 4 and above were affected by the noise floor. The broadband responses showed consistently smaller TEOAE level at the lowest stimulus levels with increasing HTL group resulting in a reduction in compression with increasing HTL group.

MLS TEOAE I/O functions were examined for differences in growth with differences in HTL. The slope of each function was calculated using linear regression, between stimulus levels of 60 and 80 dB. The median slope results for each HTL group are shown in Table 5-25.

Table 5-25: Median slope values of MLS TEOAE I/O functions (dB/dB)

	Click rate (clicks/s)					
	500				5000	
Group \ Frequency (kHz)	3	4	BB	3	4	BB
1	0.05	-0.07	0.34	0.36	-0.10	0.35
2	-0.03	-0.06	0.31	0.17	0.08	0.40
3	0.11	-0.01	0.37	0.24	0.07	0.48
4	-0.01	0.05	0.47	0.27	0.22	0.52
5	0.13	0.04	0.55	0.18	0.12	0.61
6		-0.01			0.07	

Key - BB: broadband

For the frequency-banded results, there was little or no change in compression with increasing HTL group. However, the broadband responses at both 500 and 5000 clicks/s showed a reduction in compression, and increase in I/O function slope with increasing HTL group, as predicted.

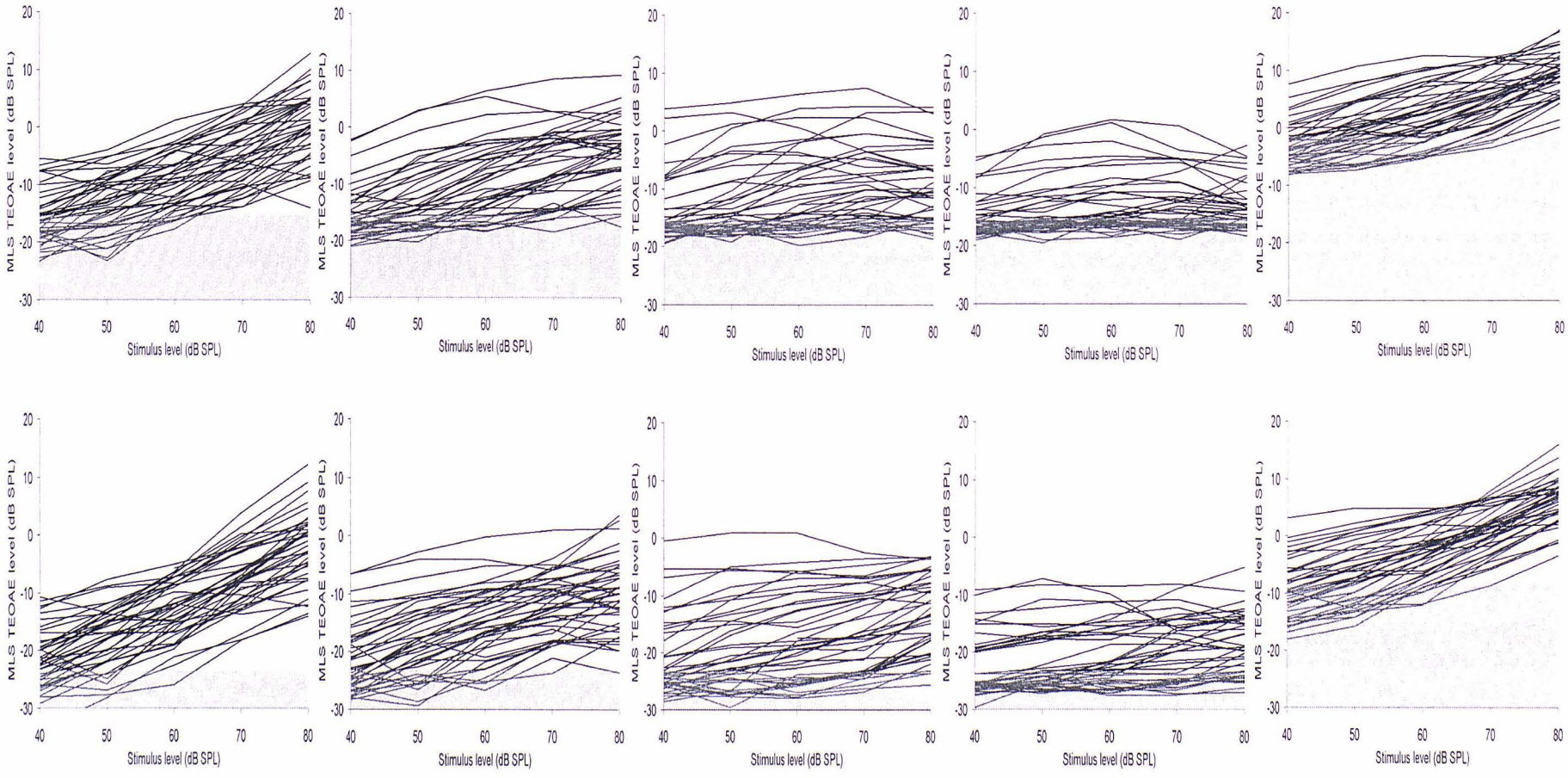


Figure 5-34: Individual MLS TEOAE I/O functions, at frequencies 1-4 kHz and for the broadband response, at click rates of 500 and 5000 clicks/s. Key. BB: broadband. Shaded area shows the mean noise floor.

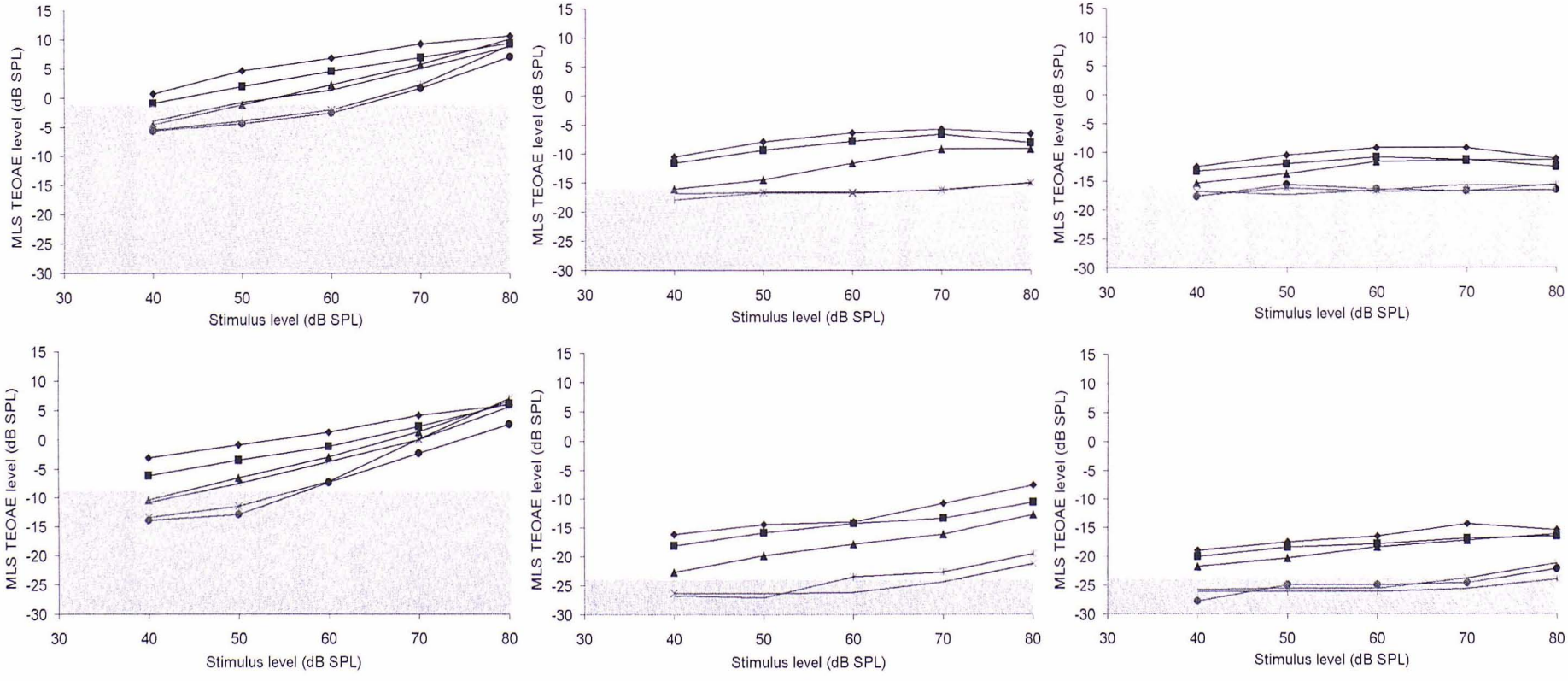


Figure 5-35: Mean MLS TEOAE I/O functions plotted by HTL group. Key Group 1: diamonds, group 2: squares, group 3: triangles, group 4: crosses, group 5: stars, group 6: circles. BB: broadband. Shaded area shows the mean noise floor.

5.6.4.3 MLS TEOAE stimulus level and HTL

The relationship between *MLS TEOAE stimulus level* and HTL was assessed in subjects with differing HTL.

MLS TEOAE stimulus level was measured for each subject. Due to the highly compressed nature of the frequency-banded results, it was not possible to estimate *MLS TEOAE stimulus level* for these subjects. However the broadband results showed sufficient growth to allow *MLS TEOAE stimulus level* to be calculated. These results are described for click rates of 500 and 5000 clicks/s. MLS TEOAE levels of 0 dB at 500 and 5000 clicks/s were used to calculate *stimulus level* as these were the lowest levels at which responses were recorded in all subjects.

MLS TEOAE stimulus levels ranged over 59 dB at 500 clicks/s, and 64 dB at 5000 clicks/s. The relationship between *MLS TEOAE stimulus level* and HTL was analysed using correlation analysis. The variables with significant correlations were then further analysed using linear regression analysis, the results of which are summarised in Table 5-26.

Table 5-26: Summary of linear regression analysis relating *MLS TEOAE stimulus level*, broadband responses (independent variable) and HTL (dependent variable)

Click rate (clicks/s)	HTL frequency (kHz)	Correlation coefficient (R)	Slope of the linear regression line
500	3	0.44***	0.36
	4	0.48***	0.53
	6	0.45***	0.63
	3-6	0.49***	0.51
5000	3	0.40**	0.39
	4	0.40**	0.51
	6	0.40**	0.66
	3*6	0.43**	0.52

*Key: * $P \leq 0.05$, ** $P \leq 0.01$ *** $P \leq 0.005$*

Statistical analysis showed significant relationships between *MLS TEOAE stimulus level* and HTL. Figure 5-36 gives an example of a significant relationship.

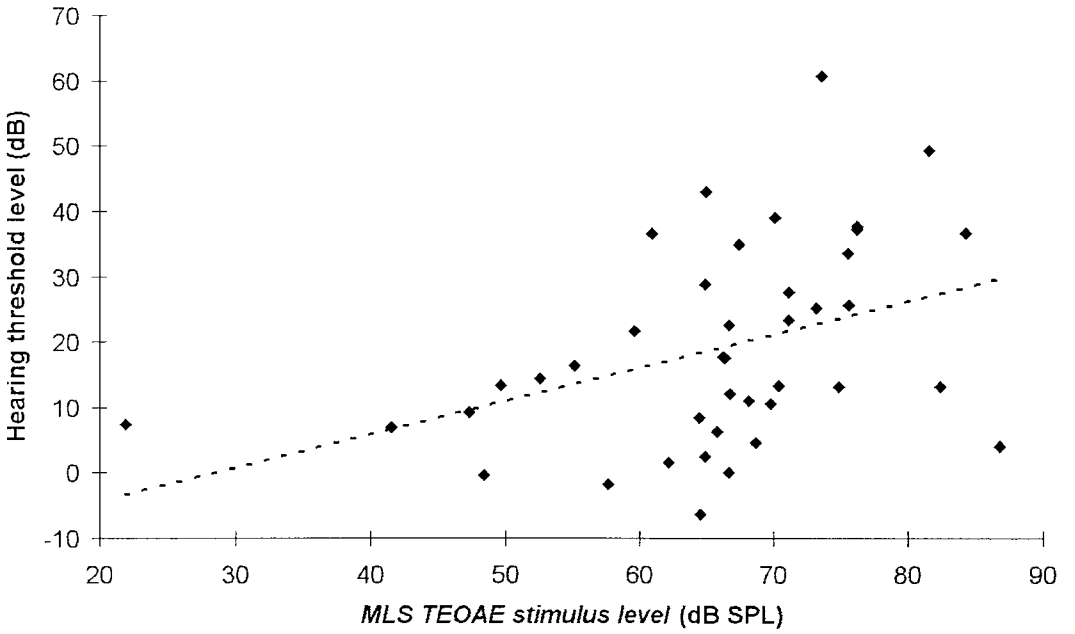


Figure 5-36: MLS TEOAE stimulus level obtained at a click rate of 5000 clicks/s, for broadband responses, plotted against 4 kHz HTL. MLS TEOAE stimulus level was the stimulus level required to evoke MLS TEOAE levels of 0 dB. Linear regression line plotted.

There was a general trend of an increase in *MLS TEOAE stimulus level* associated with an increase in HTL although there was wide variation between subjects. *MLS TEOAE stimulus level* and HTL showed the highest correlations at click rates of 500 rather than 5000 clicks/s. The strength of the correlation was similar across HTL frequencies within each click rate. However the wide variation between subjects was reflected in the low R-square values. These showed that *MLS TEOAE stimulus level* explains approximately 15-20% of the variance in HTL.

5.6.4.4 MLS TEOAE level and HTL

The relationship between MLS TEOAE level and HTL was assessed in subjects with differing HTL.

MLS TEOAE level was measured for each subject at stimulus levels that evoked MLS TEOAE above the noise floor. MLS TEOAE level varied across subjects and spanned a range of approximately 20 dB at 500 clicks/s, and up to 30 dB at 5000 clicks/s. The relationship between MLS TEOAE level and HTL was analysed using correlation analysis, the results of which are summarised in Table 5-27.

Figure 5-37 shows an example of a significant relationship. There was a general trend of decreasing MLS TEOAE level associated with increasing HTL. The highest correlations were obtained using click levels of 50-60 dB. Slightly higher correlations were obtained with MLS TEOAE evoked at a click rate of 5000 clicks/s. The highest correlations showed that MLS TEOAE level explained only 30-40% of the variation in HTL.

The correlation analysis showed a closer relationship between MLS TEOAE level and HTL than between *MLS TEOAE stimulus level* and HTL.

Table 5-27: Correlation coefficients relating MLS TEOAE level (independent variable) and HTL (dependent variable)

Click rate (clicks/s)	MLS TEOAE (kHz)	HTL frequency (kHz)	Click level (dB SPL)				
			40	50	60	70	80
500	3	3	−0.49***	−0.51***	−0.55***	−0.54***	−0.46***
			(−1.14)	(−0.97)	(−0.94)	(−0.91)	(−0.85)
		3-6 average	−0.46***	−0.50***	−0.55***	−0.58***	−0.50***
			(−1.35)	(−1.21)	(−1.21)	(−1.25)	(−1.19)
	4	4	−0.51***	−0.38*	−0.51***	−0.53***	−0.41**
			(−2.40)	(−1.34)	(−1.60)	(−1.82)	(−1.57)
		3-6 average	−0.46***	−0.32*	−0.45***	−0.47***	−0.33*
			(−2.05)	(−1.06)	(−1.35)	(−1.54)	(−1.20)
	BB	3	−0.38*	−0.44***	−0.48***	−0.39*	
			(−1.21)	(−1.15)	(−1.16)	(−1.05)	
		4	−0.38*	−0.42*	−0.50***	−0.46*	
			(−1.640	(−1.47)	(−1.62)	(−1.66)	
		6	−0.41**	−0.45***	−0.50***	−0.44***	
			(−2.29)	(−2.02)	(−2.07)	(−2.06)	
		3-6 average	−0.42***	−0.47***	−0.53***	−0.46***	
			(−1.72)	(−1.55)	(−1.61)	(−1.59)	
5000	3	3	−0.55***	−0.59***	−0.52***	−0.58***	−0.61***
			(−1.00)	(−0.92)	(−0.79)	(−0.94)	(−1.07)
		3-6 average	−0.55***	−0.62***	−0.57***	−0.61***	−0.66***
			(−1.28)	(−1.24)	(−1.09)	(−1.25)	(−1.48)
	4	4	−0.53***	−0.52***	−0.60***	−0.67***	−0.55***
			(−1.64)	(−1.46)	(−1.63)	(−1.90)	(−1.62)
		3-6 average	−0.48***	−0.47***	−0.55***	−0.64***	−0.52***
			(−1.40)	(−1.26)	(−1.49)	(−1.72)	(−1.46)
	BB	3	−0.54***	−0.61***	−0.48***	−0.33*	
			(−1.27)	(−1.40)	(−1.26)	(−0.97)	
		4	−0.48***	−0.61***	−0.50***	−0.38*	
			(−1.50)	(−1.86)	(−1.74)	(−1.53)	
		6	−0.51***	−0.60***	−0.49***	−0.42**	
			(−2.06)	(−2.39)	(−2.20)	(−2.18)	
		3-6 average	−0.54***	−0.64***	−0.52***	−0.41***	
			(−1.61)	(−1.88)	(−1.73)	(−1.56)	

Linear regression slope value in parentheses. Key: $P \leq 0.05$, ** $P \leq 0.01$ *** $P \leq 0.005$. BB broadband*

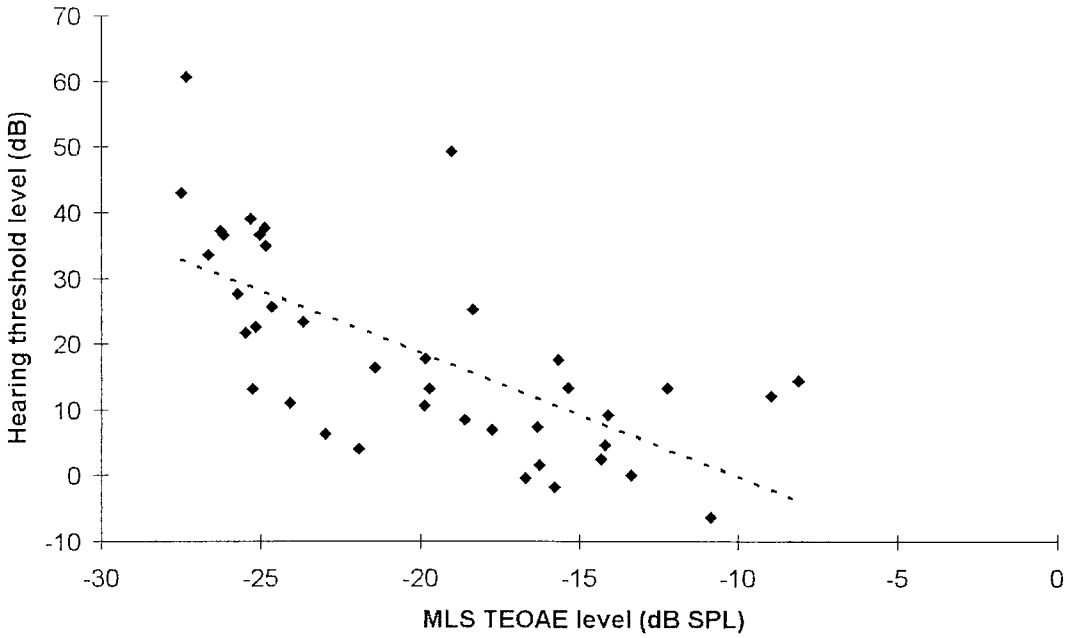


Figure 5-37: MLS TEOAE level at 4 kHz plotted against HTL at 4 kHz. MLS TEOAE level was evoked by a stimulus level of 70 dB. Linear regression line plotted.

5.6.5 MLS TEOAE rate suppression

Kapadia and Lutman (2001) proposed that the phenomenon of MLS TEOAE rate suppression is related to the nonlinear compressive properties of the TEOAE I/O function. This proposition was tested here in subjects with a range of HTL, by examining the relationship between rate suppression and the slope of the I/O function. The relationship between rate suppression and HTL was also examined.

TEOAE I/O functions obtained at 50, 500 and 5000 clicks/s all using the Natus apparatus were examined. Rate suppression was calculated as the difference in level between OAE obtained at 50 and at 5000 clicks/s (S_{5000}), and also between 50 and 500 clicks/s (S_{500}), at a particular frequency and click intensity.

The slope of the I/O function was calculated using linear regression. Two sections of the I/O function were examined: a low intensity section of the function using click levels from 40 to 60 dB, and a high intensity section between click levels from 60 to 80 dB. These ranges were chosen to investigate a region of the function where CA function was likely to be maximal (at low stimulus intensity levels), and a region where CA function was likely to be reduced (at high intensity levels).

5.6.5.1 Rate suppression at 500 clicks/s

S_{500} values were calculated at each frequency and stimulus level. MLS TEOAE responses at 4 kHz were not recordable in many subjects, so limited data are available at this frequency. I/O function

slope was calculated using the MLS TEOAE results at both 50 and 500 clicks/s. Suppression values were correlated with I/O function slope at both click rates; the significant results of the correlation analysis are shown in Table 5-28.

There were several significant relationships between rate suppression at 500 clicks/s and I/O function slope. Most were only weakly significant, and occurred for the higher frequency and broadband responses at the higher click levels. The highest correlation was obtained between rate suppression and I/O function slope when the slope of the I/O function at 500 clicks/s was used. The correlation was also higher when I/O function slope was calculated using the high stimulus section of the I/O function. Figure 5-38 shows an example of a significant relationship.

Table 5-28: Correlation between rate suppression (S_{500}) and TEOAE I/O function slope

Click rate of I/O function (clicks/s)	I/O function slope calculated between:	Frequency (kHz)	Click level (dB SPL)			
			50	60	70	80
50	40-60 dB	1				
		2	–0.53*			
		3				
		BB				
	60-80 dB	1				
		2			–0.39*	–0.39*
		3				
		BB			–0.40*	–0.56***
500	40-60 dB	1				
		2				
		3		–0.56*	–0.48*	–0.54*
		BB				
	60-80 dB	1				
		2			–0.42*	–0.73***
		3			–0.42*	–0.79***
		BB			–0.40*	–0.77***

*Key: * P=0.05, ** P=0.01, *** P=0.005. BB broadband*

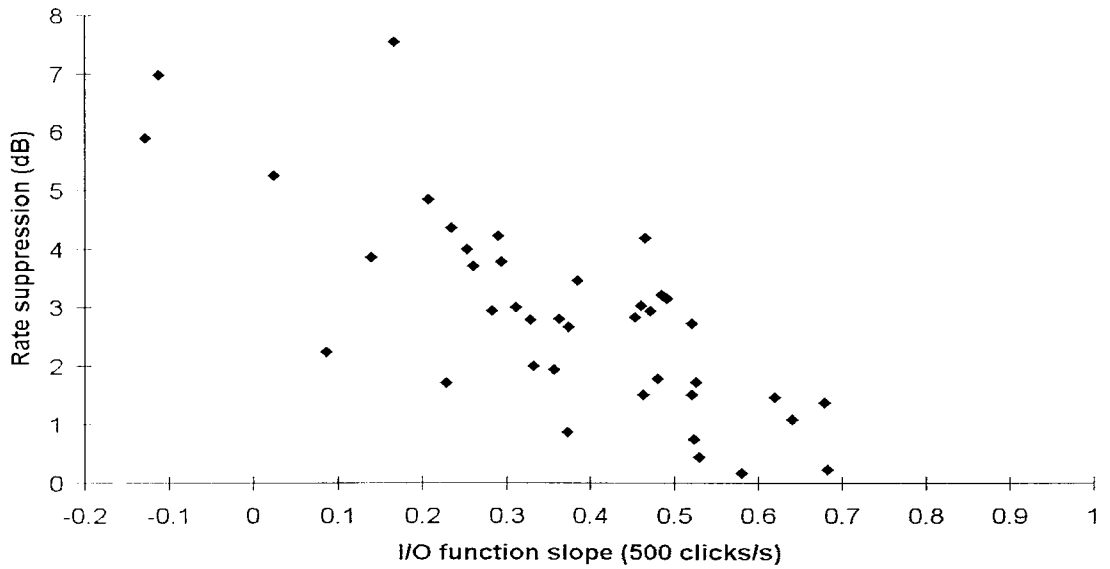


Figure 5-38: Scattergram of MLS TEOAE rate suppression plotted against I/O function slope. Rate suppression was calculated as the difference in level of the broadband response at a click rate of 50 and 500 clicks/s. I/O function slope was calculated using stimulus levels of 60-80 dB, at a click rate of 500 clicks/s.

5.6.5.2 Rate suppression at 5000 clicks/s

The analysis described for the MLS TEOAE results at 500 clicks/s was repeated using responses obtained at 5000 clicks/s. Suppression values were correlated with I/O function slope at both click rates; the results of the correlation analysis are shown in Table 5-29.

The correlation between rate suppression and I/O function slope was highly significant at most frequencies. Correlations were highest at 2, 3 kHz and for the broadband responses, with no marked differences between these frequencies. Rate suppression at 1 kHz showed the weakest correlation with I/O function slope.

The highest correlations between rate suppression and I/O function slope were obtained when suppression was correlated against I/O function slope at 5000 clicks/s; also when the slope was calculated at the higher stimulus intensity levels. As a general rule, slope values calculated at the low stimulus intensity levels had a higher correlation with rate suppression calculated at the lower intensity stimulus levels, and similarly slope values calculated at the high intensity section of the function had a higher correlation with rate suppression calculated at the higher intensity stimulus levels. Figure 5-39 shows an example of a significant correlation.

Table 5-29: Correlation between rate suppression (S_{5000}) and TEOAE I/O function slope

Click rate of I/O function (clicks/s)	I/O function slope calculated between:	Frequency (kHz)	Click level (dB SPL)			
			50	60	70	80
50	40-60 dB	1				
		2	−0.70***			
		3	−0.78***			
		BB	−0.67***			
	60-80 dB	1				
		2		−0.72***	−0.68***	
		3		−0.52*	−0.53**	
		BB		−0.70***	−0.71***	−0.44***
5000	40-60 dB	1			−0.37**	
		2		−0.69***		
		3	−0.57*	−0.78***		
		BB	−0.57*	−0.63***	−0.69***	−0.36*
	60-80 dB	1			−0.33*	−0.59***
		2			−0.69***	−0.89***
		3			−0.60**	−0.87***
		BB		−0.46*	−0.77***	−0.80***

*Key: *P=0.05, ** P=0.01, *** P=0.005. BB broadband*

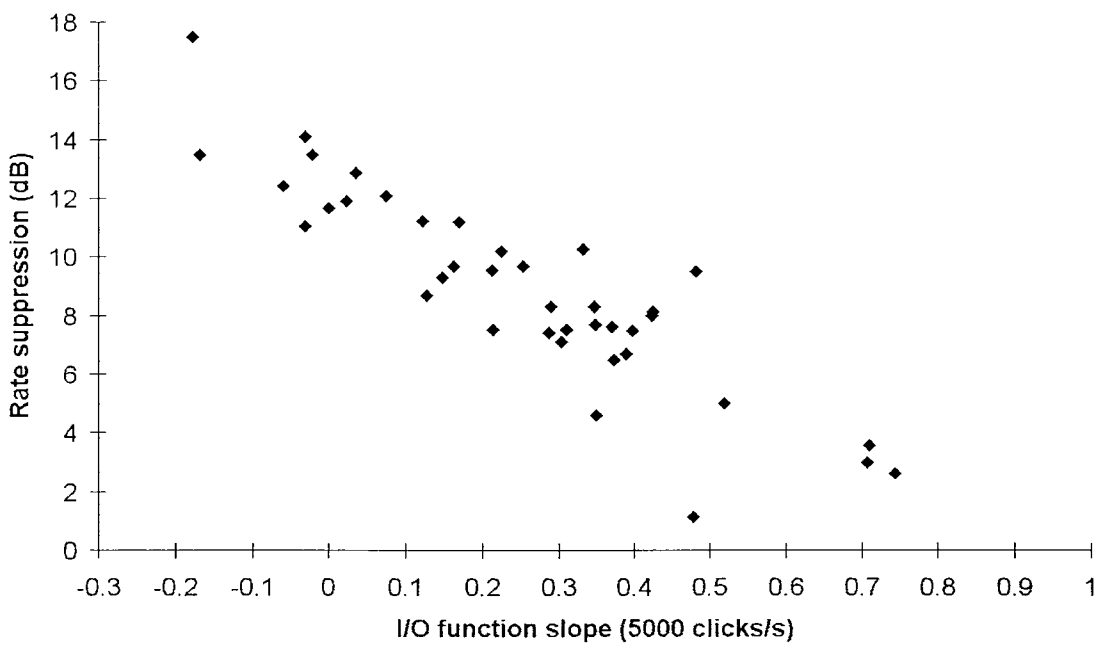


Figure 5-39: Scattergram of MLS TEOAE rate suppression plotted against I/O function slope. Rate suppression was calculated as the difference in level of the 2 kHz response at a click rate of 50 and 5000 clicks/s. I/O function slope was calculated using stimulus levels of 60-80 dB, at a click rate of 5000 clicks/s.

As the highest correlations between rate suppression and I/O function slope were observed at a click rate of 5000 clicks/s, further analysis was restricted to responses obtained at this click rate. Subjects

were then grouped according to the slope of the I/O function at 5000 clicks/s: slope values of <0.19, 0.2-0.29, 0.3-0.39, 0.4-0.49, 0.5-0.59. 0.6-0.69 and >0.7 dB/dB were used. Mean rate suppression at 80 dB was calculated for each I/O function slope group, and plotted at each frequency, see Figure 5-40. This showed a decrease in rate suppression with increasing I/O function slope. There was also a greater separation between I/O functions slopes at 5000 rather than 500 clicks/s. This was more prominent for the frequency banded results than for the broadband responses.

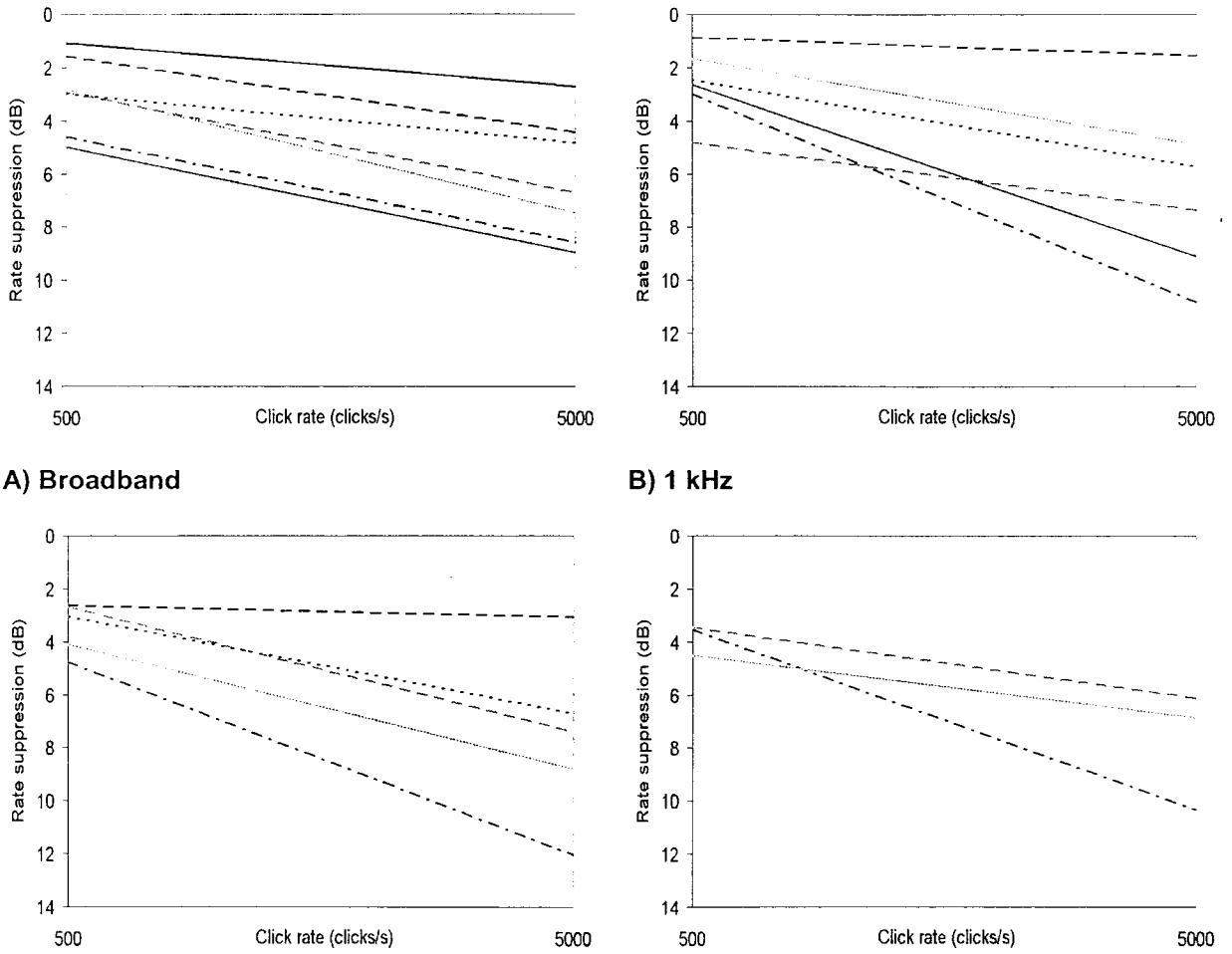


Figure 5-40: Rate suppression plotted by mean MLS TEOAE I/O function slope at click rates of 500 and 5000 clicks/s. With increasing compression of I/O function slope there is an increase in rate suppression. A) Broadband. B) 1 kHz. C) 2 kHz D) 3 kHz.

5.6.5.3 Rate suppression and HTL

The relationship between MLS rate suppression and HTL was examined using correlation analysis. Table 5-30 and Table 5-31 give the results of the statistical analysis at each frequency and click level for click rates of 500 and 5000 clicks/s. These showed significant relationships between rate suppression and HTL. Figure 5-41 gives an example of a significant relationship. There was a general relationship of increasing rate suppression with decreasing HTL, although there was wide variation between subjects. The correlation was increased at the low intensity compared to the high stimulus levels. However MLS rate suppression explained only 20-30% of the variance in HTL.

Table 5-30: Correlation coefficients relating MLS rate suppression S_{500} (independent variable) and HTL (dependent variable)

TEOAE frequency (kHz)	HTL frequency (kHz)	Click level (dB SPL)				
		40	50	60	70	80
1	3			−0.57*** (−1.98)		
	4			−0.51** (−2.35)		
	6	−0.89* (−4.89)				
	3-6 average			−0.52** (−2.25)		
2	3					−0.44** (−2.44)
	6					−0.41* (−4.01)
	3-6 average					−0.41* (−2.91)
3	4			−0.49* (−2.45)	−0.52* (−1.81)	
	6				−0.51* (−2.85)	
	3-6 average				−0.52* (−1.90)	
BB	3		−0.68*** (−3.57)	−0.52** (−3.35)	−0.42* (−3.84)	−0.54*** (−3.54)
	4					−0.51*** (−4.20)
	6					−0.38* (−4.44)
	3-6 average					−0.49*** (−4.05)

*Linear regression slope value in parentheses. Key: *P=0.05, **P=0.01, ***P=0.005. BB broadband*

Table 5-31: Correlation coefficients relating MLS rate suppression S_{5000} (independent variable) and HTL (dependent variable)

TEOAE frequency (kHz)	HTL frequency (kHz)	Click level (dB SPL)				
2	3			−0.47*	−0.38*	
				(−2.26)	(−1.84)	
	4			−0.42*		
				(−2.34)		
	3-6 average			−0.41*	−0.36	
				(−2.24)	(−2.17)	
3	3	−0.66*	−0.64*	−0.50*		
		(−1.27)	(−1.57)	(−1.85)		
	6	−0.76*				
		(−2.08)				
	3-6 average	−0.77**	−0.60*	−0.50*		
		(−1.60)	(−2.02)	(−2.44)		
BB	3		−0.54*		−0.41*	−0.37*
			(−2.75)		(−1.88)	(−1.77)
	4		−0.52*		−0.38*	−0.36*
			(−2.88)		(−2.19)	(−2.25)
	6		−0.53*			
			(−3.56)			
	3-6 average		−0.58*		−0.37*	−0.33*
			(−3.06)		(−2.12)	(−1.97)

*Linear regression slope value in parentheses. Key: *P=0.05, **P=0.01, ***P=0.005. BB broadband*

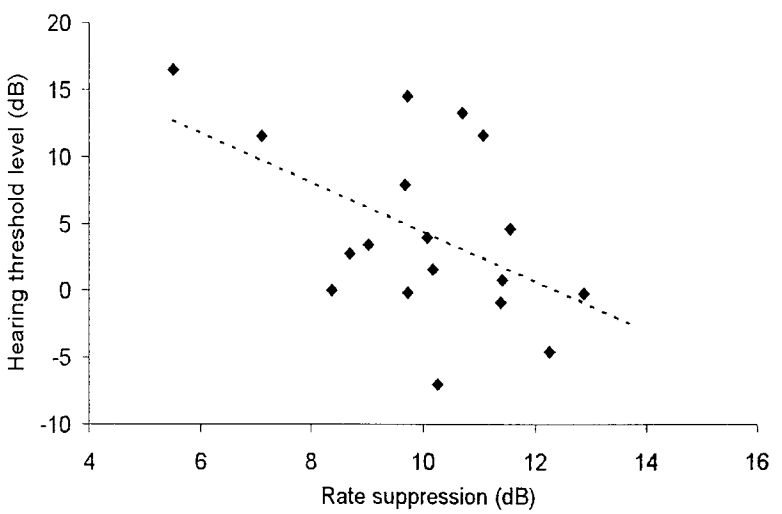


Figure 5-41: MLS TEOAE rate suppression (S_{5000}) measured at 60 dB, at a frequency of 3 kHz plotted against 3 kHz HTL. Linear regression line plotted.

5.6.6 Comparison of DPOAE and TEOAE

TEOAE and DPOAE measures were compared to evaluate commonality of origin. It was hypothesised that there would be most similarity between the different OAE measures at low stimulus levels. OAE level was examined between DP, TEOAE and MLS TEOAE. OAE *stimulus level* was not examined, as it was shown previously to be very similar to OAE level.

Correlation coefficient analysis was used to compare TE and DPOAE levels. Correlation coefficient values are shown diagrammatically in Figure 5-42.

The highest correlations between TE and DPOAE were measured at the mid-intensity stimulus levels. As there were many significant associations, attention was given to variables with correlation coefficients greater than 0.7. This showed DPOAE at 3 and 4 kHz had a close relationship with TEOAE at 2 and 3 kHz respectively, and also were significantly related to the broadband response. DPOAE at 6 kHz showed a weaker relationship with all TEOAE frequencies.

DPOAE level was then compared to MLS TEOAE level measured at 5000 clicks/s. Correlation coefficient values are shown in Figure 5-43. It was expected that the correlation coefficient values would be higher than with TEOAE level. However the coefficient values were very similar for the conventional and MLS TEOAE recording, and showed the same relationship with frequency.

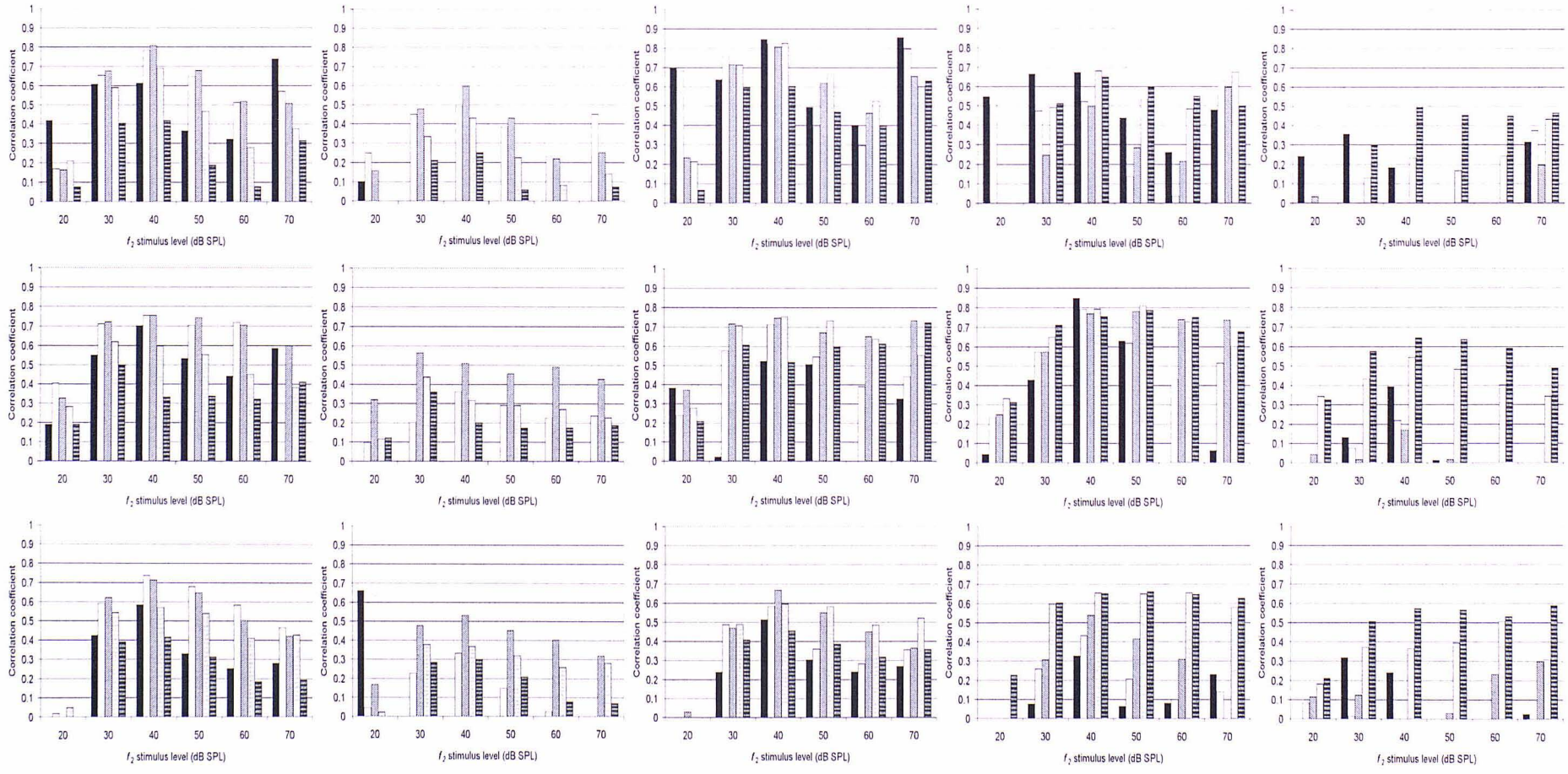


Figure 5-42: Correlation coefficients values relating DPOAE and TEOAE level at different stimulus levels. The highest correlations were between DPOAE at 3 and 4 kHz, and TEOAE at 2, 3 kHz and broadband response. Key to bars: TEOAE stimulus level – black: 50 dB, white: 60 dB, grey: 70 dB, stippled: 80 dB, horizontal stripes: 90 dB

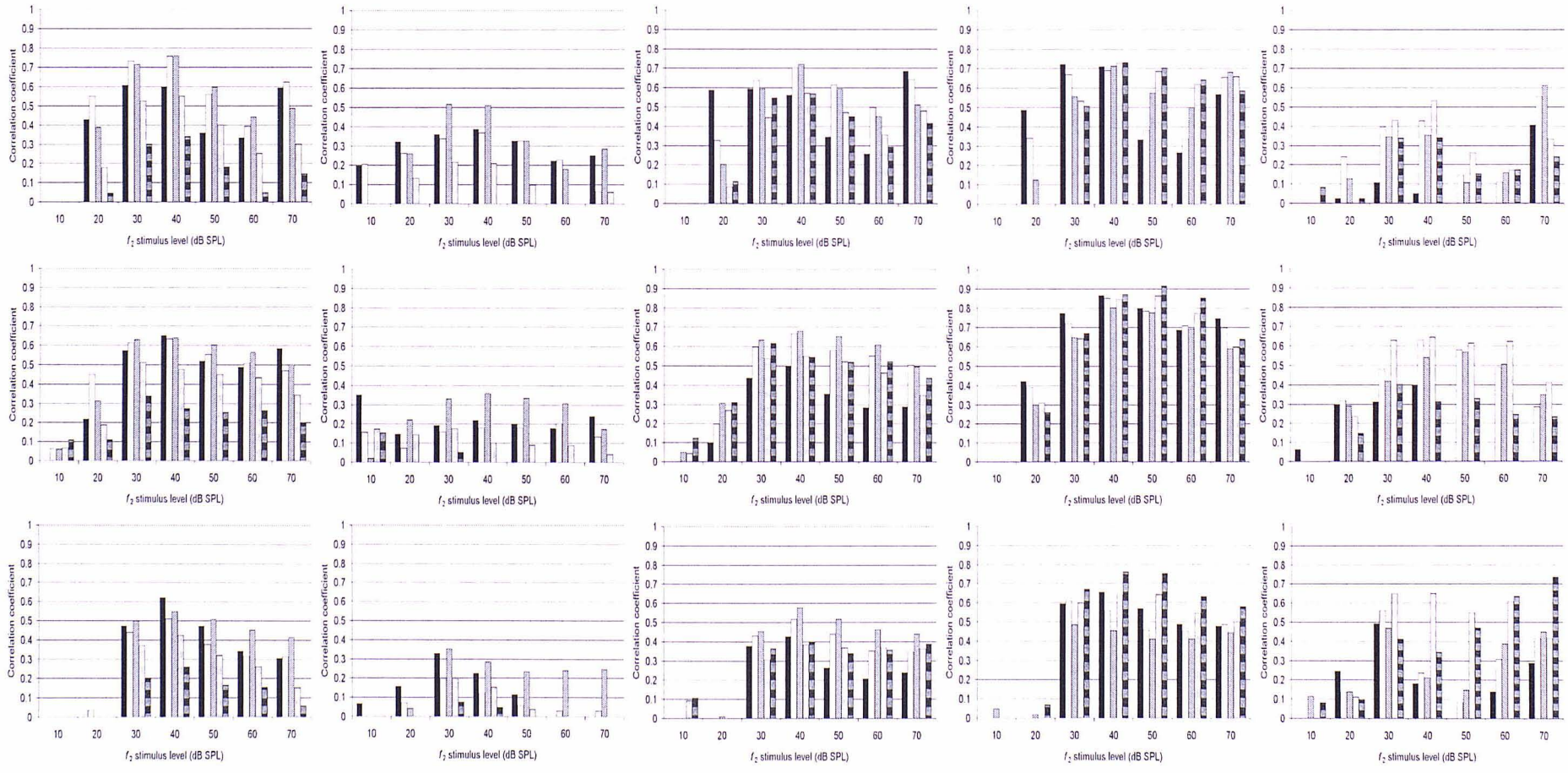


Figure 5-43: Correlation coefficient relating DPOAE level and MLS TEOAE level at different stimulus levels. These showed similar relationships to conventional TEOAE and DPOAE. Key to bars: MLS TEOAE stimulus level – black: 40 dB, white: 50 dB, grey: 60 dB, stippled: 70 dB, horizontal stripes: 80 dB.

5.7 DISCUSSION

5.7.1 Normative OAE

A library of normative values for each OAE type has been described. The effect of stimulus frequency, level and rate on OAE has also been investigated.

5.7.1.1 Conventional rate TEOAE

The normative properties of conventional rate TEOAE have been well described in the literature (Kemp et al, 1986; Probst et al, 1991) and will only be briefly discussed here. However unlike many other studies, this study recorded the entire TEOAE waveform without subtracting the linear component allowing the nonlinear component of the TEOAE to be derived at a later stage. This study was therefore able to compare properties of the linear and nonlinear derived TEOAE.

In many respects linear and nonlinear TEOAE were very similar. The nonlinear TEOAE levels were also smaller than the linear level as expected, due to subtraction of the linear component. The FFT and I/O functions derived from linear and nonlinear TEOAE had similar morphology and slope values. The repeatability of the linear TEOAE waveforms was high particularly at the early latency portions of the waveform. These results are consistent with the literature (Ravazzani et al, 1996; Gobsch and Tietze, 1997; Hatzopoulos and Martini, 1997). For these reasons, in the cross-sectional study the analysis was restricted to the linear waveforms only.

The within-session repeatability of the TEOAE waveform was high, except at a stimulus level of 40 dB. Responses obtained at this stimulus level were probably within the noise floor, particularly the lower frequency components. The between-session repeatability was not as high as within-session repeatability, but improved with increasing stimulus level.

There was wide variation between subjects in TEOAE responses. When analysed in the frequency domain, TEOAE I/O functions showed increasing compression with increasing frequency as previously reported in the literature, both for nonlinear TEOAE (Prieve et al, 1996) and linear TEOAE (Fitzgerald and Prieve, 1997). The I/O functions were not based on waveform latency it is not possible to directly compare the results to other studies of TEOAE I/O functions. TEOAE level had high repeatability both in the short- and medium-term.

5.7.1.2 MLS TEOAE

Comparison of MLS TEOAE recordings at higher click rates with recordings made at the conventional click rate showed several advantages of using higher click rates. These advantages mainly occurred at the lower stimulus levels.

There was an improvement of the within-session waveform repeatability, particularly at the lower stimulus levels. The results for a click rate of 5000 click/s gave the highest correlation. The between-session repeatability was poor at low levels, but improved with increasing click rate.

Linear waveforms were more repeatable across sessions than the nonlinear derived waveforms. Linear waveforms at 50 clicks/s also showed a better correlation to the waveforms at higher click rates than the nonlinear waveforms. These results were similar to those obtained by Thornton (1993a,b).

Increasing click rate was associated with a reduction in the noise floor. The noise floor levels for the frequency banded MLS TEOAE responses were approximately –12 dB, –16 dB and –25 dB for click rates of 50, 500 and 5000 clicks/s respectively. MLS TEOAE obtained at 5000 clicks/s therefore had the lowest noise floor of the three click rates. Waveforms at this click rate also contained more higher frequency components than the lower click rates. There was also a reduction in TEOAE level at the high click rate compared to the conventional rate.

There was no marked effect of click rate on the shapes of the I/O functions. The slopes of the I/O functions were similar across the three click rates. As for the conventional TEOAE recording there was an increase in compression with increasing frequency.

The MLS suppression results reported here, are in the main, in accordance with other studies of suppression: as reported by Hine and Thornton (1997) there was no overall effect of click level on rate suppression, although an effect of level on rate suppression was found at S_{500}, where suppression at 40 dB was significantly smaller than the other levels. At S_{5000}, rate suppression at 80 dB was significantly smaller than the other stimulus levels.

The effect of frequency on rate suppression was also investigated. There was no significant effect of frequency at S_{500}. However at S_{5000}, rate suppression at 4 kHz was significantly larger than the other frequencies. This increase in rate suppression at 4 kHz is consistent with greater compression of I/O functions measured at 4 kHz. Other studies of rate suppression have examined their data in terms of waveform latency and shown increased rate suppression with increasing latency (e.g. Lina-Granade et al, 1997), so it is difficult to compare the results of this study directly with others.

The repeatability of MLS TEOAE level was high, and this increased with increasing frequency and stimulus level. The replication SD values were approximately 1.5-2 dB at 3 and 4 kHz for all click rates used. Repeatability at the lower frequencies, particularly 1 kHz, was poorer with replication SD values of 3-4 dB. Results at 50 clicks/s were more repeatable than the higher click rates, implying that noise has a greater effect on the long latency components of the waveform from higher click rates.

5.7.1.3 DPOAE

Recording and averaging mini DP-grams gave a material improvement in DPOAE level repeatability compared to taking a single frequency measure of DPOAE level. This method did not significantly

affect level or slope of I/O function and is therefore recommended for recording DPOAE, particularly important at the lower stimulus levels where repeatability is generally poor.

DPOAE I/O functions showed wide variation between subjects and a reduction in mean compression with increasing frequency. The DPOAE I/O functions were different to the TEOAE I/O functions in that DPOAE functions showed increasing compression with a reduction in frequency, whereas TEOAE functions showed increasing compression with increasing frequency. The reasons for these differences is unclear, but may be related to the fact that the maximum energy recorded with TEOAE is at 1 to 2 kHz, and at frequencies at 4 kHz and above, TEOAE are more difficult to record (Probst et al, 1991). DPOAE however are recordable up to frequencies of 8 kHz (Probst et al, 1991).

5.7.1.4 Summary

This normative study has shown variation in OAE among normal hearing subjects. Detailed descriptions of linear TEOAE recorded using a conventional stimulus rate and also MLS are provided. Evidence is given that averaging DPOAE around the frequency of interest is preferable to measuring at one frequency in terms of repeatability. Further data are provided on MLS rate suppression, which are consistent with previous studies.

Extensive data are provided on the short- and medium-term repeatability of OAE.

5.7.2 Cross-sectional study

OAE measures were investigated in a cross-sectional study of human subjects. They are discussed separately below for each OAE type.

5.7.2.1 DPOAE

DPOAE I/O functions have been used for examining cochlear function in human neonates (Abdala et al, 1999; Abdala, 2000 and 2001a, b) and adults (Kummer et al, 1998; Janssen et al, 1998; Dorn et al, 2001). The present study used Mills' model of DPOAE I/O functions to develop a framework for use in human subjects.

It was possible to transfer the principles of Mills' model of DPOAE I/O functions to human subjects. Human DPOAE I/O functions had a similar morphology to those obtained to low-level stimuli in gerbils, and differences in DPOAE I/O functions between subjects with differing HTL were similar to the changes observed in gerbil I/O functions undergoing a reduction in CA gain. However it is important to remember that gerbils do not have any material reflection from f_{dp}, whereas humans do.

With increasing HTL, DPOAE I/O functions showed a greater reduction in level at the lower intensity stimulus levels compared to the higher stimulus levels. This resulted in a reduction in compression that is consistent with a reduction in CA function. This was observed across each of the frequencies tested.

Interestingly, the differences observed in the DPOAE I/O functions in this study were not related to small differences in HTL. There appeared to be two main categories of DPOAE I/O function that were broadly related to normal hearing and mild-to-moderate hearing impairment. Subjects with normal hearing typically had I/O functions that were compressive, with large level DPOAE across the stimulus intensity level. Subjects with hearing impairment typically had functions with reduced compression and smaller level DPOAE. According to studies of BM vibration, a reduction in CA gain results in reduced sensitivity to low intensity stimuli, and increased linearity in the growth of vibration with stimulus level (Robles and Ruggero, 2001). This was similar to the DPOAE I/O functions recorded in this study from subjects with hearing impairment.

DPOAE I/O functions obtained from subjects with normal hearing (ranging from –10 to 20 dB HL) were of similar morphology and although the hearing levels of these subjects covered a 30 dB range there were no significant differences between subjects with differences in HTL within this range. This is consistent with Mills' results, which did not show small changes in CA gain using DPOAE I/O functions, only large changes.

DPOAE level growth at low DPOAE levels was approximately 1 dB/dB in all subjects. Other studies of human adult DPOAE I/O functions have reported similar results (Kummer et al, 1998). A growth of 3 dB/dB was not recorded in any of the subjects in this experiment, although in some subjects, growth approached a value of 2 dB/dB at 6 kHz. A slope of 3 dB/dB is expected when the CA saturates at high levels, or when the CA gain is zero and DPOAE generation is from purely passive, mechanical properties of the cochlea. However this was not observed in this experiment. Neither did Mills in his experiment record DPOAE growth at values approaching 3 dB/dB. The maximum reported growth was 2 dB/dB.

There could be several reasons that a growth rate of 3 dB/dB was not recorded here. Technological limitations may have contributed to the inability to record DPOAE to lower intensity levels than currently possible, where a higher growth rate may be recorded (Nelson and Zhou, 1996). Alternatively it may be that even when the gain of the CA is zero, other nonlinearities may contribute to DPOAE generation and a growth rate of 3 dB/dB does not occur physiologically.

The mechanism of passive generation of DPOAE is unclear. Mom et al (2001) induced ischaemia in gerbils. They recorded DPOAE I/O function slopes of between 2.2 and 3.2 dB/dB in the post-ischaemic animals. This provides evidence for the existence of passive DPOAE, and shows that slopes of 3 dB/dB can be recorded experimentally. However Mom et al (2001) showed that these supposedly "passive" DPOAE obtained to high intensity stimulus levels were still physiologically vulnerable to noise. This implies that their origin was not as "passive" as initially thought. They speculated that the sensitivity of high intensity stimulus DPOAE depends on the nature of the physiological insult to the cochlea, and that different pathologies may affect the cochlea in different

ways. Changes in the mechano-electric transduction (MET) processes of the OHC affect high intensity DPOAE, but other changes do not (e.g. ischaemia causing the EP to drop). Mom et al (2001) surmised that reduction in DPOAE level at high stimulus levels as well as low levels was related to a direct change in OHC motility. If this was not observed, then they concluded the feedback pathway was intact. If high intensity level DPOAE are sensitive to insult, it is unlikely that a slope of 3 dB/dB will be recorded.

The results of the correlation between DPOAE level and HTL showed significant correlations with DPOAE evoked by stimulus levels of 70 dB. This implies that DPOAE at these stimulus levels were not evoked simply by passive generation. If they were of purely passive origin then it is expected that there would be no difference in DPOAE level between subjects with differing HTL. It is possible that insufficiently high stimulus levels were used in this experiment to evoke passive DPOAE, as limited by the equipment.

The relationship between the DPOAE measures and HTL showed significant correlations. The relationship between *DPOAE stimulus level* and HTL gave maximum R-square values of approximately 0.6. This indicates that only 60% of the variation in HTL can be explained by DPOAE. DPOAE level gave similar results to *DPOAE stimulus level* and these were similar to the results of other studies reported in the literature (e.g. Kim et al, 1996). The highest correlations between DPOAE level and HTL were measured using low to moderate stimulus levels of 40, 50 and 60 dB SPL. These also gave maximum R-square values of approximately 0.60 for the relationship between DPOAE level and HTL.

It was hypothesised here that the use of new technology to record DPOAE might allow DPOAE to be recorded at lower signal to noise ratio, and thus improve the correlation between DPOAE and HTL as compared to previous studies. The average noise floor values recorded in this study ranged from –22 dB SPL at 3 kHz down to –35 dB SPL at 6 kHz. These values are lower than the noise floor in some the earlier studies investigating the relationship between DPOAE and HTL, such as Nelson and Kimberley (1992), whose noise floor values ranged from approximately 0 to –10 dB SPL across the frequency range. The values are slightly lower than some later studies, mainly at the higher frequencies, such as Kummer et al (1998), where the mean noise floor at 6 kHz was approximately –20 dB SPL. However they are similar to those reported by Gorga et al (1994), where the mean noise floor was approximately –30 dB SPL at frequencies of 500 to 4 kHz. However, the low noise floor of the recordings of this study did not markedly improve the correlation coefficients recorded in this study, and they were similar to others reported in the literature (see Table 2-4 for references).

It was shown in the normative study that recording mini DP-grams improved the repeatability of DPOAE I/O functions. However taking cochlear fine structure into account by recording mini DP-

grams to calculate the DPOAE measure did not increase the correlation with HTL compared to previous studies.

Comparison of the correlation coefficient results of this study to those of Pienkowski and Kunov (2001) showed similar values. Pienkowski and Kunov (2001) investigated an alternative method using DPOAE STC to measure CA function as proposed by Mills (1998). The relationship of the DPOAE STC measure and HTL gave R-square values of approximately 0.5. This was lower than the maximum R-square value obtained using DPOAE level in this study. It could be argued that the DPOAE STC method is superior to the DPOAE I/O function method because the measure calculated from DPOAE STC gives an absolute value that is not relative to another subject. It also has the advantage of cancelling out between-subject factors, which may account for some of the variability in this experiment. However using DPOAE STC gave results that have a similar relationship with HTL to other methods.

The hypothesis that DPOAE measures based on a framework of cochlear amplification would result in higher correlations with HTL than previous studies was not upheld. The DPOAE measures investigated in this study showed similar correlation values to those reported in the literature. DPOAE evoked using lower stimulus levels had a higher correlation with HTL than those evoked to higher stimulus levels, but the results of this study still showed that approximately 40% of the variation in HTL was unaccounted for and 60% was explained by DPOAE.

There are several possibilities to explain these results. Recent work by Mills (Mills, 2002) has discredited the notion of "passive" and "active" DPOAE in animals, and the original model (Mills, 1997) is now questioned. The original model was based on contributions to the DPOAE arising from two slightly different locations of the basilar membrane, one at high stimulus levels and one at low stimulus levels, which is now thought to be false. From the model, Mills predicted a relationship between the shift in DPOAE and CA gain. The moderate relationship between DPOAE and HTL shown in this study may be result of the deficiencies in the model recently described.

Although Mills' model was based on gerbil ears, it was felt to be a useful framework for human subjects. However it may be inappropriate for humans, particularly as it does not account for the distortion/reflection generation mechanism of DPOAE. Although the main component of DPOAE generation in humans is thought to be through distortion generated at the f_2 site (at a frequency ratio of 1.2) (Knight and Kemp, 2000), taking into account the reflection component at the $2f_1 - f_2$ site may be important in other studies of the correlation between DPOAE and HTL. Shaffer et al (2003) suggest that for future studies investigating the relationship with HTL, it may important to take into account the multiple generation sites of DPOAE. The framework based on Mills' model did not take account frequency from multiple sites, and this is therefore likely to be important for future models.

The unexplained variance in the relationship between DPOAE and HTL between subjects and between ears may be due to differences in ear canal size, middle-ear transmission etc. A longitudinal experiment investigating changes within subjects is required to investigate this further.

Alternatively the main assumption of the thesis, that OAE and HTL are closely linked through the CA, may be false. As stated as an alternative to this assumption, other factors apart from the CA may also be important in the relationship between DPOAE and HTL, affecting them in different ways. There may be differences in the aetiology of the hearing impairments used in the study; some subjects may have IHC dysfunction affected HTL but not DPOAE generation. Also some subjects may have minor cochlear abnormalities affecting DPOAE, but not HTL.

A second experiment is required in which the aetiology of hearing impairment is the same across subjects, and within-subject and within-ear factors are controlled for.

5.7.2.2 TEOAE

Although TEOAE are generally not recorded with HTL greater than 30 dB HL, the aim of this experiment was to investigate differences in I/O functions within the range of hearing thresholds at which TEOAE are evoked, a range of 40 dB from -10 to 30 dB HL.

The morphology of the TEOAE I/O functions with increasing HTL was different from that predicted from the framework. The frequency-banded TEOAE I/O functions at 3 and 4 kHz showed considerable overlap between the HTL groups, and there were no consistent differences in the I/O functions with increasing HTL. On the other hand, the broadband TEOAE I/O functions were similar to the predicted framework. They showed a reduction in TEOAE level at the lowest stimulus intensity levels, giving an increase in the slope of the I/O function with increasing HTL group number. There were minor or no differences in TEOAE level at the highest stimulus levels.

The reason why the results were different from the original framework may be related to the generation mechanisms of TEOAE. Mills' model relies on the largest changes in OAE occurring at low intensity levels and little or no changes at the high levels. It is acknowledged that there is no "passive" TEOAE generator, but with a reduction in cochlear active mechanisms, growth of TEOAE is predicted to increase towards 1 dB/dB. At this point active involvement is minimal, and distortion mechanisms may be involved in TEOAE production. This increase in growth or reduction in nonlinearity with increasing HTL was observed for the broadband results, with a slope of 0.4 dB/dB in the normally hearing groups increasing to 0.8 dB/dB in the mild hearing loss group. Growth in the mild hearing loss group approached the theoretical 1 dB/dB value of a "passive" case, and was consistent with studies of BM vibration. No subjects had growth rates much greater than 1 dB/dB.

However at the frequencies of 3 and 4 kHz, TEOAE I/O functions were compressed across the HTL groups, with little or no reduction in compression with increasing HTL. At 3 kHz, growth of TEOAE

level over moderate intensity stimulus levels varied from 0.3 dB/dB in the normally hearing groups up to 0.4 dB/dB in the mild hearing loss group. At 4 kHz, TEOAE growth reached a maximum of 0.3 dB/dB indicating compression across the normal hearing HTL groups. There were no data available for comparison with the mild/moderate HTL groups. One of the difficulties of examining TEOAE I/O functions in subjects with HTL up to 30 dB HL is that subjects with mild hearing losses tend to only generate TEOAE at the higher stimulus levels only. This meant it was not possible to plot I/O functions for all subjects.

Even though the range of HTL examined in this experiment spanned 40 dB, there were no consistent differences in the frequency banded (3 and 4 kHz) TEOAE I/O functions within this range of hearing. However the broadband TEOAE I/O functions were consistent with the framework.

The fact that the frequency-specific TEOAE did not follow the predicted pattern and the lack of data at the low stimulus intensity levels meant that it was difficult to apply the method for estimating differences in TEOAE I/O functions. Due to the pattern of the TEOAE I/O functions, it was difficult to calculate *TEOAE stimulus level* for a pre-set level in all subjects. Approximately half did not have TEOAE present at stimulus levels of 80 dB or below, although most had a TEOAE at 90 dB. However it was possible to analyse the broadband results in this way.

The hypothesis that TEOAE measures based on a framework of cochlear amplification would result in a higher correlation with HTL than previous studies was not upheld. The correlation between TEOAE measures and HTL was low at 3 kHz and there was no significant correlation at 4 kHz. The broadband TEOAE measures showed the highest correlation with HTL, although this may because more subjects elicited a broadband TEOAE response. The maximum R-square values were 0.3, showing that *TEOAE stimulus level* explained only 30% of the variation in HTL.

In contrast to the method of calculating *TEOAE stimulus level*, using TEOAE level was easier to apply to all subjects. This showed significant correlations between TEOAE level and HTL. The broadband TEOAE level showed the highest correlation with HTL and this was greatest at the lowest stimulus levels. The maximum R-square values that were obtained were approximately 0.4, showing that TEOAE level explained only 40% of the variation in HTL. This was a slight improvement on *TEOAE stimulus level.*

It was predicted that TEOAE measures based on the framework of TEOAE I/O functions would show a higher correlation with HTL. However TEOAE level showed a higher correlation with HTL than *TEOAE stimulus level.* This implies that the *TEOAE stimulus level* method is not advantageous for conventionally recorded TEOAE and that TEOAE level is preferable.

It was also predicted that TEOAE evoked to lower level stimuli would show a higher correlation with HTL. This was true for the broadband responses, where TEOAE evoked to 60 and 70 dB stimuli had higher correlation with HTL than TEOAE evoked to 80 dB stimuli.

The difficulty of applying the framework to TEOAE I/O functions requires a re-assessment of the framework. It was based on cochlear amplification having a greater input to TEOAE at low stimulus levels, and the invulnerability of TEOAE at high intensity levels. If this was the case, it is expected that all subjects would have similar TEOAE levels at high stimulus levels. However this was only observed for the broadband responses. This implies that high level TEOAE may not be invulnerable or that the variation arises from some other factor. Cochlear amplification may still be involved in generation of TEOAE at the highest stimulus level, and thus TEOAE at high levels are sensitive to small differences in HTL between subjects. The mixture of nonlinear distortion and reflection at high level TEOAE (Yates and Withnell, 1999) may also account for the deviation of the experimental results from the framework. The framework did not specify frequency, and in the same way as suggested for DPOAE (Shaffer et al, 2003) it may be necessary to take into account other frequencies, particular if distortion at high frequencies contributes to TEOAE at low frequencies as proposed by Avan et al (1997).

As discussed for DPOAE, the fundamental assumption of a relationship between TEOAE and HTL mediated by the CA may be flawed. There may be differences in TEOAE between subjects resulting from differences in reflection sites along the cochlea that are unrelated to cochlear amplification. A second experiment is required to investigate further whether the relationship between HTL and OAE is improved when changes within subjects are followed.

5.7.2.3 MLS TEOAE

Much of the discussion for TEOAE also applies to MLS TEOAE. This section concentrates on discussion regarding the application of the MLS technique to TEOAE.

MLS TEOAE I/O functions were plotted for responses obtained at click rates of 500 and 5000 clicks/s. The MLS TEOAE I/O functions obtained at 500 and 5000 clicks/s showed similar morphology. As frequency increased, the functions showed more compression. They showed more compression than the conventional TEOAE.

As was observed for the conventional TEOAE I/O functions, the measured MLS TEOAE I/O functions at 3 and 4 kHz were not consistent with the predicted framework. At both frequencies MLS TEOAE I/O functions were highly compressed, and showed no increase in level with stimulus level. I/O functions from HTL groups with mild hearing loss or above were all below the noise floor, and functions from the other groups were all similar and showed considerable overlap. The mean slope values of the 500 clicks/s I/O functions in the normal hearing groups ranged between 0 to 0.1 dB/dB at 3 kHz. At 4 kHz, the mean slope values were approximately 0 dB/dB across all the HTL groups. At 5000 clicks/s the slope values were slightly higher and ranged between 0.2 dB/dB to 0.3 dB/dB at 3 kHz. At 4 kHz, slope values were lower and ranged from 0 dB/dB up to 0.2 dB/dB.

The broadband MLS TEOAE I/O functions were consistent with the predicted framework. The MLS technique enabled responses to be measured at stimulus levels of 40 and 50 dB in most of the HTL groups, and at these lowest stimulus levels there was good separation between the HTL groups. With increasing HTL group number there was a reduction in compression, which was evidenced by an increasing slope of the I/O function. This was most discernible at the click rate of 5000 clicks/s, where the mean slope values ranged from 0.2 dB/dB at the lowest HTL groups up to 0.7 dB/dB in the mild hearing loss group. This result was not observed so clearly for the conventional TEOAE responses, and is therefore a potential advantage of the MLS technique.

It was hypothesised that using the maximum length sequence recording technique would enable TEOAE responses to be measured to lower signal-to-noise ratios than with conventional recording, and therefore that MLS TEOAE would be more sensitive to differences in HTL between subjects than conventional TEOAE. It was therefore predicted that MLS TEOAE would show a higher correlation with HTL than the conventional TEOAE, particularly at the lower intensity stimulus levels where conventional TEOAE were unable to record responses.

The highly compressed nature of the I/O functions at 3 and 4 kHz meant that it was difficult to measure *MLS TEOAE stimulus level* and this could not be applied at these frequencies. The method was applied to the broadband responses, and *MLS TEOAE stimulus level* was correlated with HTL. *MLS TEOAE stimulus level* showed moderately significant correlations with HTL. Responses obtained at 500 clicks/s had higher correlation coefficients than those obtained at 5000 clicks/s. The maximum R-square value obtained was 0.2, indicating that only 20% of the variation in HTL is explained by *MLS TEOAE stimulus level.*

MLS TEOAE level was also investigated and showed a significant correlation with HTL, which was higher than the correlation between *MLS TEOAE stimulus level* and HTL. Results obtained at a click rate of 5000 clicks/s showed a higher correlation with HTL than those obtained at 500 clicks/s, particularly at the lower intensity stimulus levels. The correlation with HTL was similar to that obtained using conventional TEOAE. MLS TEOAE responses were obtained at lower stimulus levels than was possible with conventional TEOAE, and these responses evoked by low intensity stimuli also showed a significant correlation with HTL. The maximum R-square value that was obtained was 0.45, indicating that only 45% of the variation in HTL is explained by MLS TEOAE level.

Comparison of the two different MLS TEOAE methods shows that using MLS TEOAE level is an easier method to apply than *MLS TEOAE stimulus level.* This is likely to be related to the highly compressive I/O functions of MLS TEOAE, particularly at the high frequencies that make it difficult to apply this method.

Although the noise floor measured with the MLS TEOAE recording technique was approximately 10 to 20 dB lower than the conventional TEOAE recording, the correlation of MLS TEOAE measures with HTL was actually slightly lower the conventional TEOAE measures with HTL.

The advantage of the MLS technique is that it enables TEOAE to be recorded in subjects with higher HTL than in the conventional recording method. This was most prominent at the higher frequencies and low intensity stimulus levels and is likely to be a result of averaging a larger number of responses, enabling smaller level responses to be detected above the noise floor. This also enabled responses to be detected in more subjects with mild hearing loss than possible with conventional TEOAE.

Contrary to the prediction, the use of MLS technology to record TEOAE did not materially improve the correlation between TEOAE and HTL. However the fact that responses were obtained at lower stimulus levels in more subjects than with conventional recording methods is useful. The literature suggests that TEOAE evoked by low intensity levels are more sensitive to change, and a longitudinal experiment investigating changes in TEOAE is required.

5.7.2.4 MLS rate suppression

The model relating MLS rate suppression and the nonlinearity of the I/O function was tested, and the results were generally consistent with the model proposed by Kapadia and Lutman (2001). Rate suppression and the slope of MLS TEOAE I/O function showed highly significant correlations, and provide further evidence to support a cochlear source of rate suppression (Hine et al, 1997) rather than an efferent source (Lina-Granade et al, 1997).

The relationship between rate suppression and I/O function nonlinearity was dependent on frequency, click level and click rate. The highest correlations were obtained using a click rate of 5000 clicks/s and a click level of 80 dB. Rate suppression under these stimulus conditions showed the highest correlation with I/O function nonlinearity measured at a click rate of 5000 clicks/s, with the function slope calculated at the high intensity stimulus levels. The highest correlations were obtained when rate suppression and I/O functions at the same frequency were examined, at 2 and 3 kHz and for the broadband response. Interestingly, there was no significant relationship between I/O function slope and rate suppression at 1 kHz.

The relationship between suppression and I/O function nonlinearity was associated with stimulus level. Suppression calculated at the lower intensity levels had a greater correlation with the slope of the I/O function when calculated at the lower part of the function. Suppression at the higher levels showed a greater correlation with the slope calculated at the higher part of the function.

The high correlation between TEOAE rate suppression and I/O function nonlinearity provides further evidence for a cochlear source to rate suppression. It is likely that the same cochlear mechanisms are responsible for the nonlinearity of the I/O function as for rate suppression. Increasing rate leads to a

superimposition of the stimulus with the MLS, giving larger BM amplitude. This is equivalent to the I/O function. The lack of a relationship at the low frequencies may be related to properties of the apical cochlea, which are different to the basal end. Results described in this study showed I/O functions at low frequencies were more linear than at other frequencies.

It was hypothesised that because rate suppression measures are strongly related to I/O function nonlinearity and therefore likely to be related to CA function, they would have a high correlation with HTL. However the MLS TEOAE rate suppression measure showed a weak correlation with HTL. Rate suppression obtained at 500 clicks/s had a slightly higher correlation with HTL than the 5000 clicks/s measure. The broadband responses showed the highest correlations. The maximum R-square value obtained was 0.45 indicating that rate suppression explained only 45% of the variation in HTL.

Although there is good evidence that rate suppression is cochlear on origin, and likely to be related to OHC activity, the measure did not have a higher correlation with HTL than other OAE measures. This maybe because other nonlinearities, apart from at the site of cochlear amplification are involved in rate suppression. Kapadia and Lutman (2001) discuss the possibility that between-channel nonlinearities may be important. Alternatively it may be because the assumed relationship between HTL and OAE, mediated through the CA is false.

A longitudinal experiment is required to determine whether the relationship is improved when changes within subjects are examined.

5.7.2.5 Comparison of DPOAE and TEOAE

Comparison of DPOAE and TEOAE level showed the highest correlation coefficients at the mid-frequencies and at the mid-intensity stimulus levels. The highest correlations were measured between TEOAE at 2 kHz and DPAOE at 3 kHz, and TEOAE at 3 kHz and DPOAE at 4 kHz, with DPOAE stimulus levels of 40 dB and TEOAE stimulus levels of 50-70 dB. Correlation coefficients in these cases were 0.8 and above. These results were compared to those of Knight and Kemp (1999), who evaluated the relationship between DPOAE and TEOAE at 2 kHz for a variety of DPOAE parameter settings and distortion product frequencies. Their correlation analysis was performed between the $2f_1 - f_2$ DPOAE with TEOAE evoked at a stimulus level of approximately 70 dB SPL, using an f_2/f_1 frequency ratio of 1.2. They reported high correlations between DPOAE and TEOAE at the lower DPOAE stimulus levels. This is similar to the results in this study at 2 and 3 kHz.

There were low correlations in this study between TEOAE frequencies of 1 and 4 kHz with all DPOAE frequencies, both at the highest and lowest stimulus levels. The lowest recorded DPOAE frequency in this experiment was 3 kHz, so the large frequency separation between DPOAE and

TEOAE at 1 kHz may explain the low correlation at these frequencies. It is not known why TEOAE at 4 kHz did not show high correlations with the higher DPOAE frequencies.

The correlations between DPOAE and MLS TEOAE were similar to that between DPOAE and the conventional rate TEOAE responses. However the MLS TEOAE recorded at the lower stimulus levels had higher correlations with DPOAE than the conventional TEOAE at the same stimulus levels. This is to be expected as MLS TEOAE responses were measured at lower signal to noise ratio than conventional TEOAE, and thus measured responses at lower stimulus levels. The high correlation of the two OAE types at the mid-frequencies indicates similarity in generation mechanisms at these frequencies. It remains to be seen whether this similarity between subjects is also measured in the changes within subjects.

5.8 CONCLUSIONS

The main hypothesis tested in this cross-sectional study was that the OAE measures investigated in this thesis would show a higher correlation with HTL than those previously reported in the literature. OAE measures were chosen to account for the level dependency of the cochlear amplifier, and low level stimuli and high stimuli rates were examined. Equipment that enabled measurement to low noise floor was used.

Although OAE measures evoked to low stimulus levels had higher correlations with HTL than those evoked to high stimulus levels, the correlations were no greater than previous reported in the literature (e.g. Gorga et al, 1993a, b; Kim et al, 1996; Dorn et al, 2001). The use of new technology for recording DPOAE and MLS TEOAE enabled responses at low signal-to-noise ratios to be measured, however these were also no greater. MLS TEOAE rate suppression showed a very high correlation to the nonlinearity of the I/O function, suggesting a similar cochlear origin for the two processes. However MLS TEOAE rate suppression did not have a high correlation with HTL.

Therefore the hypothesis that the OAE measures investigated here would show a higher correlation with HTL than described in the literature was not upheld. The results showed that OAE does not fully explain differences in HTL between human subjects and that the different OAE measures explained only up to 60% of the variance in HTL. Of the OAE measures examined, DPOAE showed the highest correlations with HTL. DPOAE measured using lower level stimuli had a higher correlation with HTL than those measured using high levels. A possible reason is that DPOAE is more representative of cochlear amplification than TEOAE. Alternatively, as DPOAE were evoked from more hearing impaired subjects than for TEOAE, there was a greater spread of data across the HTL range, which may have improved the correlation analysis. The data from TEOAE, which were only evoked in normal hearing subjects and those with a mild hearing loss, were concentrated at one end of the HTL range and this may have resulted in lower correlations. Comparison of all OAE data from normal hearing subjects showed equally wide variation.

A further experiment is required to determine whether inter-subject/ear factors unrelated to cochlear amplification (e.g. ear canal and middle-ear factors) are contributing to the moderate relationship between OAE and HTL. This will involve examining changes within subjects undergoing a temporary hearing threshold shift and investigating whether changes in OAE have a higher correlation with changes in HTL than the cross-sectional differences measured in Experiment 1.

6 EXPERIMENT 2: LONGITUDINAL STUDY

6.1 AIMS

Experiment 2 aimed to explore the relationship between the OAE measures and HTL by inducing a temporary hearing threshold shift in human subjects using aspirin.

6.2 OBJECTIVES

The main objective was to characterise the longitudinal changes in cochlear function due to aspirin consumption over a period of seven days in terms of changes in OAE and HTL

Several of the objectives were the same as for Experiment 1, but were longitudinal in nature. These were to explore measures of OAE in subjects developing and recovering from a temporary hearing threshold shift.

A further objective was to compare and contrast the longitudinal changes in cochlear function (OAE, HTL) to the results of the cross-sectional study. Finally this study set out to compare the changes in DP and TEOAE from aspirin.

6.3 HYPOTHESES

The assumption of this thesis is that there is a close relationship between OAE and HTL due to the commonality of cochlear amplification for OAE generation and acute hearing sensitivity. It is hypothesised that this relationship is obscured because of inter-subject differences that influence OAE and HTL differently. It is therefore expected that longitudinal changes in OAE and HTL will have a higher correlation than the cross-sectional differences in OAE and HTL reported for Experiment 1.

The alternative hypothesis is that inter-subject differences make little contribution to the variation in both OAE and HTL, and if this is correct, the relationship between OAE and HTL is expected to be no greater than in the cross-sectional study.

Specific hypotheses tested are:

1. OAE I/O from human subjects with changing HTL will reflect the framework of OAE I/O functions. Increasing HTL will be associated with increasingly linear I/O functions. The changes

within subjects will be more consistent with the framework than the differences between subjects. Changes in HTL will be reflected by:

   a. OAE I/O function nonlinearity

   b. OAE level at a pre-set stimulus level

   c. OAE stimulus level at a pre-set OAE level

2. OAE measures will reflect changes in HTL, as hypothesised for Experiment 1. The changes in the OAE measures will have a higher correlation with changes in HTL than the differences in OAE with differing HTL

3. TEOAE will reflect changes in HTL better than DPOAE due to their primarily reflection source generation mechanism. For this reason, aspirin will have a greater effect on TEOAE than on DPOAE level. This will be dependent on stimulus parameters, and TEOAE evoked by lower level stimuli are hypothesised to be most sensitive.

4. Within-subjects, there will be a relationship between the change in TEOAE and DPOAE. For example, subjects that show the largest shifts in TEOAE will have the largest shifts in DPOAE.

6.4 REVIEW OF SALICYLATE AND HEARING

Salicylates were chosen as the means for inducing a temporary hearing threshold shift in this experiment. Aspirin (a type of salicylate) is a common, over-the-counter drug that is used therapeutically for pain relief and as an anti-inflammatory agent. Two of the side effects of this drug are reversible tinnitus and hearing loss. For this reason it is often used to induce temporary hearing threshold shift in hearing research experiments and was used for this purpose in this thesis. The pharmacokinetics and the effects of aspirin on hearing are reviewed briefly below.

6.4.1 Pharmacokinetics

The chemical name for aspirin is acetylsalicylic acid. Within the blood, it is hydrolysed to the pharmacologically active ion, salicylate; 80-90% of salicylate is carried in the blood in loose association with a plasma protein (Levy, 1979). This is known as bound salicylate. Free (non protein-bound) salicylate is thought to be the most pharmacologically important and is known as unbound salicylate.

Salicylate is metabolised in the liver and primarily excreted by the kidneys. The pharmacokinetics of salicylate are dose-dependent, and the pathway of its breakdown and elimination in the body is summarised in Figure 6-1.

Of the five elimination products, two exhibit nonlinear elimination kinetics (shown in Figure 6-1). The enzymes involved in elimination of these products become saturated at plasma levels greater than 100 μg/ml. So, at low plasma concentrations (50-60 μg/ml), salicylate has a half-life in the body of 2-3 hours. At the levels used in chronic pain relief, the half-life can be between 12-30 hours. This has implications when considering dose regimes, as increasing the concentration of the drug gives a more than proportional increase in the steady-state level of salicylate in the body. With increasing dose, the elimination rate of salicylate also decreases, and therefore the time to reach steady state increases. (Levy et al, 1972; Levy, 1979). This also has implications when designing experiments using high doses of aspirin in terms of when to start the aspirin regime, and how to measure the blood concentration.

The effect of aspirin dose on salicylate plasma concentration is highly variable between subjects and hence there is little correlation between these two variables (Day et al, 1989). A single pain relief dose of aspirin gives plasma salicylate concentrations of approximately 30 μg/ml. When used regularly for chronic pain relief, the concentration is higher at 150-300 μg/ml (Day et al, 1989).

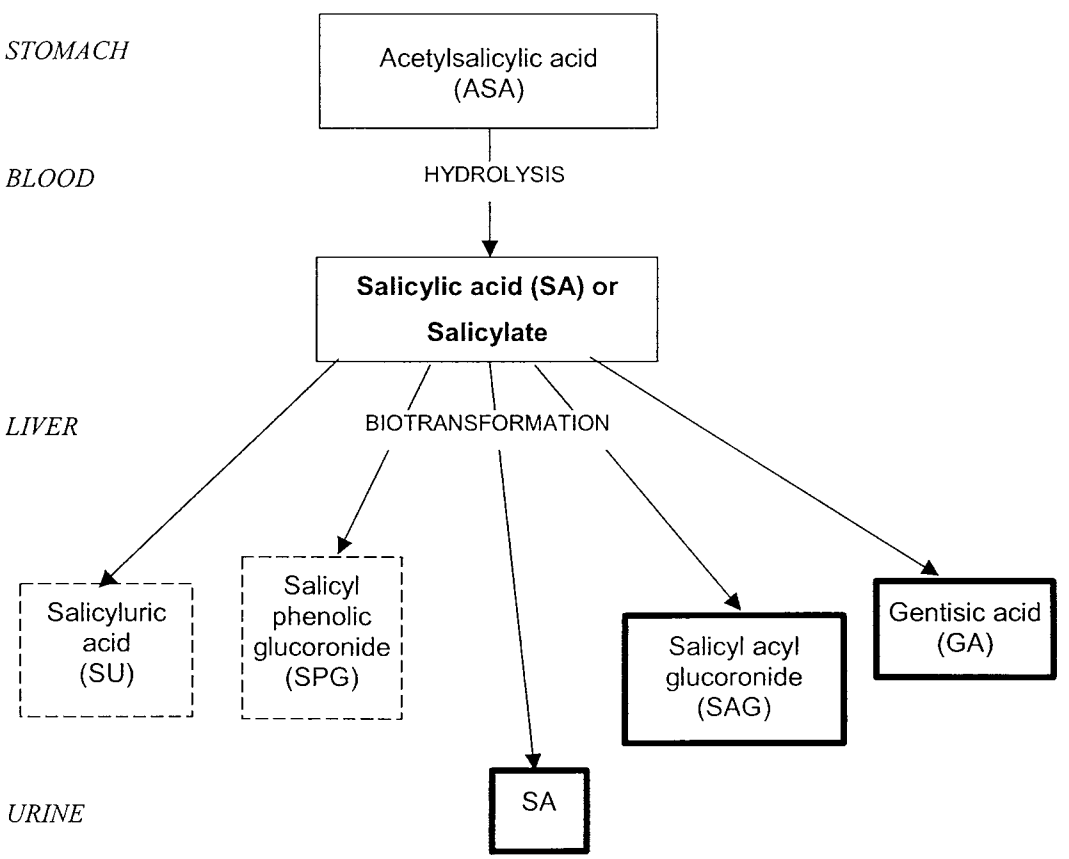


Figure 6-1: The pathways of the elimination of aspirin in the body (adapted from Levy, 1972). Key: dose dependent kinetics: dashed line boxes, 1st order kinetics: thick lined boxes.

6.4.2 Ototoxicity

Salicylate permeates most tissues and fluid compartments, including the cochlea. Most evidence points towards salicylate acting on the OHC within the cochlea. Perfusion of salicylate on isolated OHC has been shown to cause a reduction in hair cell turgor. Douek et al (1983) analysed the cochlear hair cells of guinea pigs injected with salicylate to identify any structural changes. They reported swelling of the membranous structures within the cochlea, which included both inner and OHC. The OHC were affected to a greater degree. This change in OHC was also demonstrated by Shehata el al (1991) who showed that the reduction in turgor gave a reduction in cell electromotility thus diminishing the cochlear mechanical tuning of the basilar membrane. They also showed an increase in OHC membrane conductance, although the increase in conductance was dissociated with the change in turgor and electromotility.

Stypulkowski (1990) investigated the physiological effects of salicylate on the hearing of cats. The effect of salicylate was assessed on the action potential, the cochlear microphonic, nerve fibre tuning curves, DPOAE level and efferent stimulation. Salicylate caused a reduction in action potential level, which was greatest at the lower intensity stimulus levels. There was also an elevation and broadening of the nerve fibre tuning curves. DPOAE level was reduced with salicylate. These changes are all related to a change in the mechanical amplification process of the OHC, particularly evidenced by the change in action potential level only at low intensity stimuli levels. There was also a slight increase in cochlear microphonic level indicating a change in OHC conductance. These changes were all very similar to those changes induced with efferent stimulation. Stypulkowski (1990) concluded that salicylate had two effects: increasing OHC conductance and reducing the mechanical amplification properties of the OHC (i.e. a reduction in cochlear amplifier gain). He did not specify a linkage between these two effects.

Mechanical studies of the effect of salicylate on basilar membrane movement were performed by Mammano and Ashmore (1993) and Murugasu and Russell (1995), also indicating an OHC effect.

6.4.3 Effect of salicylate on hearing threshold level

Salicylate can cause a reversible increase in HTL that may also be accompanied by tinnitus. Ototoxic effects are observed at salicylate plasma levels as low as 150 μg/ml (Myers et al, 1965; Mongan et al, 1973). Mongan et al (1973) performed an experiment to determine the salicylate plasma level at which tinnitus was induced in groups of rheumatoid arthritis patients and also in healthy, normal hearing subjects. In the normal hearing group, tinnitus was induced at levels ranging from 245-380 μg/ml. In the RA patients, this was at levels of 196-458 μg/ml.

There is a strong correlation between unbound salicylate in the plasma and HTL. HTL increases with increasing unbound salicylate concentration until it plateaus at a level of approximately 40 dB HL, at which point there is no further increase in HTL with further increase in salicylate concentrations (Day et al, 1989). This is consistent with disablement of the cochlear amplification mechanism.

There are large inter-subject differences in the effect of aspirin on hearing. This may be due to variation in the absorption of salicylate between subjects (Day et al, 1989). Bilateral, symmetrical threshold shifts of 10-50 dB have been shown depending on frequency. The loss does not tend to exceed 50 dB. Table 6-1 summarises the salicylate dosages and accompanying hearing threshold shifts that have been reported in the literature.

Research has shown that salicylate is more likely to influence frequencies of normal hearing rather than impaired regions (Myers et al, 1965). However Carlyon and Butt (1993) reported that it tends to affect all frequencies, although other studies reported that higher frequencies are affected first (McCabe and Dey, 1965). Original thresholds were generally returned to within 24-72 hours after the last dose.

Other studies have investigated the effect of salicylate on psychophysical measures of hearing. Carlyon and Butt (1993) measured the effect of aspirin on equivalent rectangular bandwidth. They showed an increase in filter width at 4 kHz for most subjects with salicylate consumption. Beveridge and Carlyon (1996) also recorded a similar increase in filter width with salicylate. Such an increase in equivalent rectangular bandwidth is consistent with a reduction in tuning curves, likely as a result from OHC dysfunction. For a detailed review of the effect of salicylate on hearing, refer to Cazals (2000).

6.4.4 Aspirin as a model for sensorineural hearing loss

The effects of salicylate on hearing strongly suggest an effect at the OHC level of the cochlea, and indicate a reduction in the active mechanisms in the cochlea. A sensory hearing loss of up to a maximum of 40-50 dB irrespective of frequency has been shown. Salicylate is therefore a suitable tool for reducing the gain of the cochlear amplifier in experiments requiring induction of a sensory hearing loss.

In order to assess compliance of any subject taking part in such an experiment, it is important to measure plasma salicylate concentration. The pharmacokinetics described earlier are important for this reason, and also for designing the optimal drug regime to ensure maximum salicylate concentration in the blood.

Table 6-1: Summary of aspirin dosages and corresponding hearing threshold shifts reported in the literature

No.	Study	Number of subjects	Dose per session (g)	No sessions per day	Total daily dosage (g)	Number of days	Total dosage (g)	Hearing threshold shift (dB)	Frequencies affected
1	McCabe and Dey (1965)	5	NS	NS	5	4	20	8 to 27	HF more than LF
2	Myers and Bernstein (1965)	25	NS	NS	6 to 8	Until subjective effects	Varied	20 to 40	Normal hearing frequencies
3	Pedersen (1974)	14	NS	NS	4	3 to 4	12 to 16	0 to 40	All frequencies
4	Young and Wilson (1982)	5	1.5	Varied	6 to 8	1 to 7	Varied	0 to 20	HF
5	McFadden and Plattsmier (1983)	4	0.975	4	3.9	2.75	10.725	5 to 19	Larger change at 3.5 kHz compared to 2.5 kHz
6	McFadden and Plattsmier (1984)	5	0.975	4	3.9	3.75	14.625	–5 to 20	All frequencies
7	McFadden et al (1984)	5	0.975	4	3.9	5	19.5	10 to 20	-
8	Long and Tubis (1988)	4	0.975	4	3.9	3 to 4	11.7 to 5.6	0 to 20	-
9	Wier et al (1988)	4	0.975	4	3.9	4	15.6	–6 to 9	All frequencies
10	McFadden and Champlin (1990)	5	0.975	4	3.9	4	15.6	5 to 16	-
11	Brown et al (1993)	8	0.96	4	3.84	2	7.68	2 to 30	All frequencies
12	Carlyon and Butt (1993)	8	0.96	4	3.84	2	7.68	0 to15	All frequencies
13	Beveridge and Carlyon (1996)	9	0.96	4	3.84	3	11.52	–1 to 12	-

Key – HF: high frequencies, LF: low frequencies, NS: not stated

6.5 METHODOLOGY

6.5.1 Subjects

A power analysis estimated the numbers of subjects required to detect various group mean hearing threshold shifts with 80% power using estimates of test-retest variability obtained from Experiment 1. Table 6-2 shows the number subjects required for each temporary threshold shift (TTS). As there is wide variation in the TTS generated using aspirin, the conservative estimate of a 5 dB mean hearing threshold shift was chosen. This gave a requirement for 18 subjects.

Table 6-2: Results of the power analysis showing the number of subjects required with differing effect sizes (significance level; p=0.05; power 80%)

Hearing threshold shift (effect size) (dB)	Within-subject standard deviation of threshold shift (dB)	Number of subjects required
5	5	18
7.5	5	9
10	5	6
15	5	4

In total, nineteen male subjects with a mean age of 27 (age range 19 to 38 years old) were tested. Female subjects were excluded to avoid known cyclic variation in hearing thresholds and OAE due to hormonal factors, and also possible complications that may arise from taking aspirin relating to bleeding and menstruation. All subjects received a medical and audiological screen prior to taking part in the experiment.

6.5.2 Medical screening

Subjects were examined medically by Mr M Pringle, FRCS, Consultant ENT surgeon at the ISVR Hearing and Balance Centre, University of Southampton, to screen out those people at risk of side-effects from aspirin. The GP of each subject was also notified by post of the nature of the experiment and given two weeks to respond if they felt there were any contra-indications to their patient taking part in the experiment.

Subjects who suffered from the following disorders were excluded from the study: asthma, hay fever, urticaria, any gastro-intestinal disorder (including ulcers), gout, impaired liver function, vitamin K

deficiency, haemophilia, nasal polyps; if they regularly took diuretics, had undergone recent surgery, or had a clinically important illness within four weeks prior to the start of the experiment; if they had a history of hypersensitivity to aspirin or other anti-inflammatory drug; if they had high blood pressure or were generally unhealthy. As subjects were required to have a small amount of blood taken, they were excluded if they had any infectious diseases that can be transmitted through blood, such as hepatitis B or HIV. The medical screening form is shown in Appendix 6.

6.5.3 Audiological screening

Subjects all had air and bone conduction HTLs better than or equal to 20 dB at all frequencies. Bone conduction thresholds were within 5 dB of air conduction thresholds averaged across 0.5, 1, 2 and 4 kHz. All had normal middle-ear function as determined by otoscopy and tympanometry (middle-ear compliance between 0.3 and 1.5 ml, middle-ear pressure between –50 and +50 daPa). An audiological questionnaire was used to determine whether there was any significant noise exposure, balance problem or ear disease (Appendix 4). If so, subjects were excluded from taking part in the study. A tinnitus rating scale was also applied to assess the presence of distracting tinnitus in subjects prior to the experiment. This involved a thermometer scale ranging from 0 to 100 where 0 was no tinnitus and 100 was the worst tinnitus possible (Appendix 7). As one of the side effects of aspirin is temporary tinnitus, those with distracting tinnitus pre-aspirin consumption (denoted as 50 or above on the thermometer scale) were excluded from the study.

6.5.4 Aspirin dosage

The aim of the experiment was to administer the maximum safe dosage of aspirin to generate the greatest TTS possible. The daily dosage was set just below the maximum recommended for self-administration by the British National Formulary of 4 g and also to be consistent with previous TTS studies using aspirin. Previous temporary hearing threshold shift experiments using aspirin consumption have demonstrated hearing threshold shifts at individual frequencies from approximately –10 to 30 dB (Cazals, 2000). Figure 6-2 summarises the range in HTL shifts obtained for varying dosages of aspirin across a range of experiments.

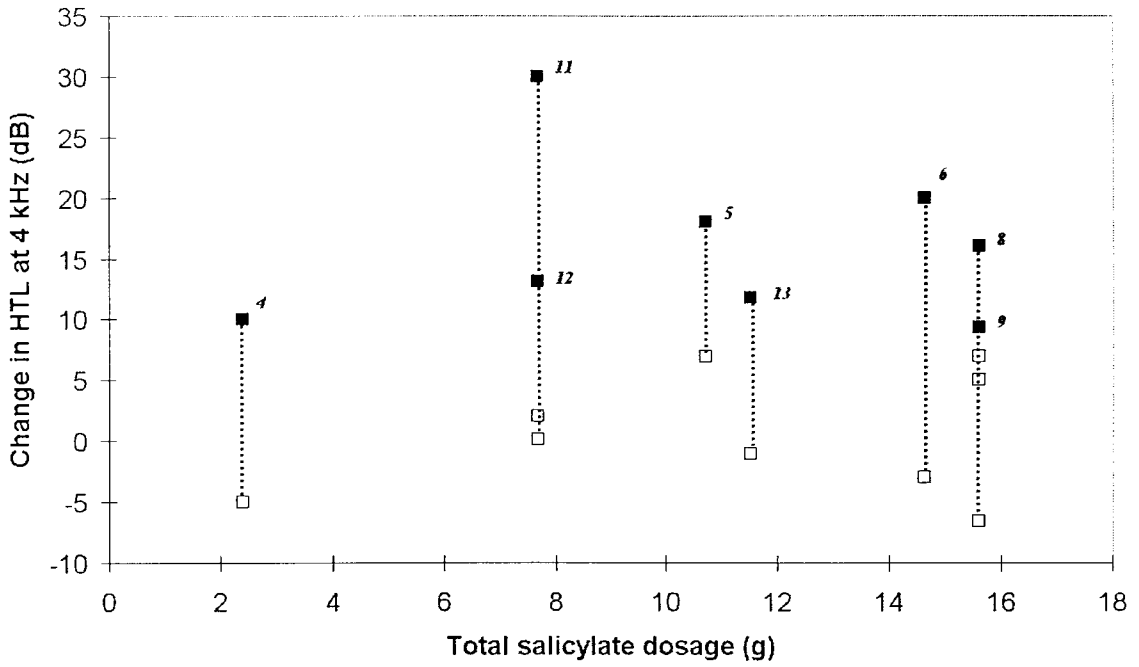


Figure 6-2: Summary of total salicylate dosage and the change in HTL reported in the literature. Key: minimum change in HTL: open square, maximum change in HTL filled square. Numbers refer to the studies quoted in Table 6-1.

A drug regime was chosen that was similar to other studies in the area. This involved a daily dosage of 3.9 g aspirin over a course of three days. Subjects took 975 mg of aspirin (three 325 mg capsules) every four hours for 3 days according to the test schedule shown in Table 6-3. These dosage intervals have been shown to be sufficient to maintain plasma salicylate levels at the desired concentration (Levy, 1979).

Aspirin capsules were manufactured specifically for the project through the Southampton General Hospital Pharmacy department, which verified the dosage and contents of the capsules.

The dosage regime was designed so that the first two doses were taken on the Sunday, ensuring that at session 3 the subjects would be starting to reach the plateau of maximum salicylate concentration within the blood stream. It was important to give time for this to occur as outlined in Section 6.4: at the higher dose regimes the time to reach steady state within the blood stream is longer.

To facilitate correct dosage, subjects were given a dose box containing the number of aspirin tablets to be taken at each session on each day for the course of the experiment. They also filled in a diary to confirm their intake of aspirin. Any subject who reported substantial side effects (e.g. troublesome tinnitus or stomach upset) or experienced TTS greater than 25 dB was immediately withdrawn from the experiment.

Table 6-3: Daily test schedule and aspirin dosage

Clinic session	Day	Test interval	Aspirin dosage and times
1	*	Pre-aspirin	Nil
2	*	Pre-aspirin	Nil
*	Sunday	Peri-aspirin	975mg @ 16:00 & 20:00
3	Monday	Peri-aspirin	975mg @ 08:00, 12:00, 16:00 & 20:00
4	Tuesday	Peri-aspirin	975mg @ 08:00, 12:00, 16:00 & 20:00
5	Wednesday	Peri-aspirin	975mg @ 08:00 & 12:00
6	Thursday	Post-aspirin	Nil
7	Friday	Post-aspirin	Nil

* *Not specified.*

6.5.5 Subject instructions

Informed consent to participate in the experiment was obtained from all subjects. Subjects were asked to read an information sheet explaining the side effects (Appendix 8). They were warned that they might suffer from minor side effects such as mild stomach upsets and tinnitus. They were also warned of the potential risk of serious side effects such as gastro-intestinal haemorrhage, increased pulse rate, sweating, dizziness, hyperventilation, fainting. They were informed that if they experienced any of these symptoms they should stop taking aspirin immediately.

Subjects were told to avoid exposure to excessive noise levels (e.g. rock concerts, shooting) during the experiment, as aspirin is known to increase the temporary hearing loss caused by exposure to loud noises. They were advised to take the aspirin with a full glass of water, and to consume a small snack with each dose, in order to minimise the possibility of an upset stomach. They were also told to refrain from alcohol consumption whilst taking the drug and not to consume more than the four-hourly three-tablet dose. They were required to refrain from taking any other non-prescribed drug during the course of the experiment and were all given a 24-hour contact telephone number.

At the start of each session, subjects were asked to complete the tinnitus severity rating scale. This was necessary to identify subjects who were experiencing strong tinnitus effects from the aspirin. Any subject who rated the severity of their tinnitus greater than 50% was withdrawn from the study. At

session 7, subjects were asked to complete a post-test questionnaire, see **Appendix 9**. This was used to document whether the subject had experienced any side effects from the aspirin.

Subjects were paid £10 per session completed and an extra payment of £30 on completion of the experiment.

6.5.6 Blood analysis

Plasma salicylate concentrations were monitored in each subject by analysis of blood samples; 300 µl of blood was obtained from each subject by the researcher at sessions 3, 4 and 5 for analysis of total plasma salicylate concentration to assess subject compliance with the aspirin regime.

Due to constraints specified by the ISVR Safety and Ethical Committee, it was not always possible to obtain a blood sample (e.g. if it was later than 5 pm and the ISVR first aider was not present, or if the specified room for blood taking was unavailable). For these reasons plasma salicylate results are not available for all subjects on each day; however most subjects gave at least two samples over the course of the experiment. The pharmacological analysis was performed at the Southampton General Hospital pharmacology department using an enzymatic assay.

6.5.7 Audiological measures

All audiological measures were obtained at sessions 1 through to 7 as detailed in Table 6-3. Each session lasted approximately 2 hours. Both ears of each subject were tested to maximise the data collected and also to assess any differential ear effect of aspirin.

Sessions 1 and 2 were used to establish baseline measures and assess test-retest repeatability of the measures. It was important to establish a reliable baseline, as the changes that were likely to occur would be small in most cases. Sessions 3, 4 and 5 measured the peri-aspirin effect, and sessions 6 and 7 measured the post-aspirin effect. HTL values were compared to pre-aspirin values and monitored until they returned to original values.

Due to time constraints, there were some slight differences in this experiment compared to the protocol for Experiment 1. No recordings were taken at 40 dB stimulus level for both MLS and conventional TEOAE. Results from Experiment 1 had shown that most subjects had OAE below the noise floor at this stimulus level.

Also it was only possible to measure MLS TEOAE using two out of three of the click rates described in the general methods section. Click rates of 50 and 5000 clicks/s were chosen, based on the results of Experiment 1. Analysis of the OAE rate suppression results showed the largest rate suppression occurred between click rates of 50 and 5000 clicks/s rather than 50 and 500 clicks/s. For this reason 500 clicks/s was omitted from Experiment 2.

OAE measures were calculated as for Experiment 1. However the method of calculating *OAE stimulus level* was not used, as the results of Experiment 1 showed they were very similar to the results obtained using OAE level.

6.6 RESULTS

6.6.1 Missing data

Of the nineteen subjects tested, two subjects (numbers 5 and 18) experienced side effects from the aspirin. Both subjects were withdrawn from the study and did not complete the full aspirin regime; for this reason their results are not included within the analysis. The remaining 17 subjects completed the full dosage regime, although two subjects missed one test session each.

6.6.2 Plasma salicylate

The blood salicylate assay showed that all subjects were compliant with the aspirin regime. Figure 6-3 shows the individual results.

There was wide variation between subjects in plasma concentration of salicylate. Salicylate concentration in general increased from session 3 to session 5, with the greatest concentration measured, on average at session 5. Of the group, ten subjects achieved a plasma salicylate concentration of greater than 1 mmol/l on at least one session.

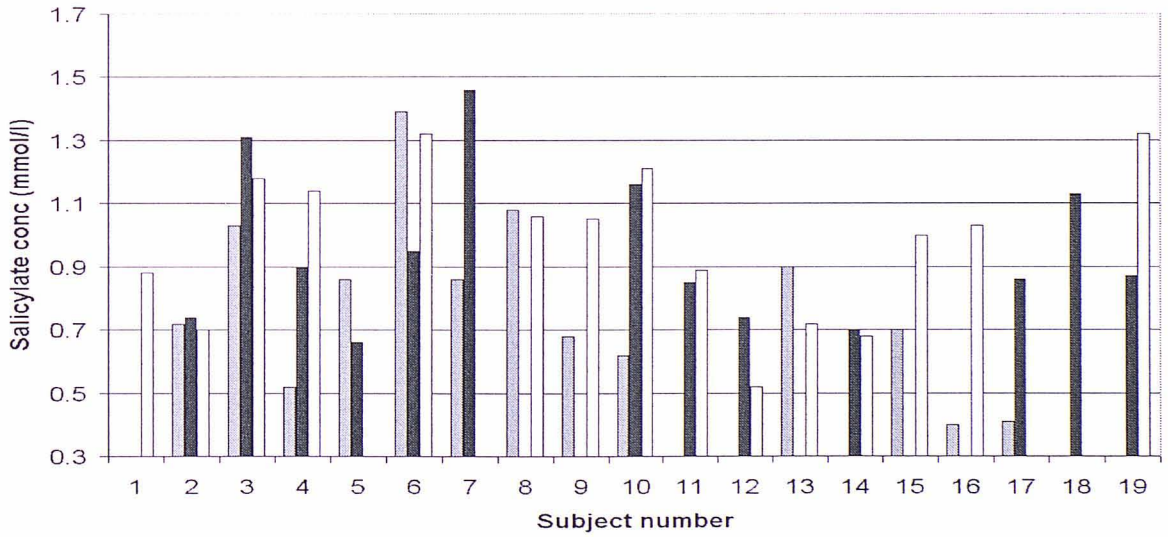


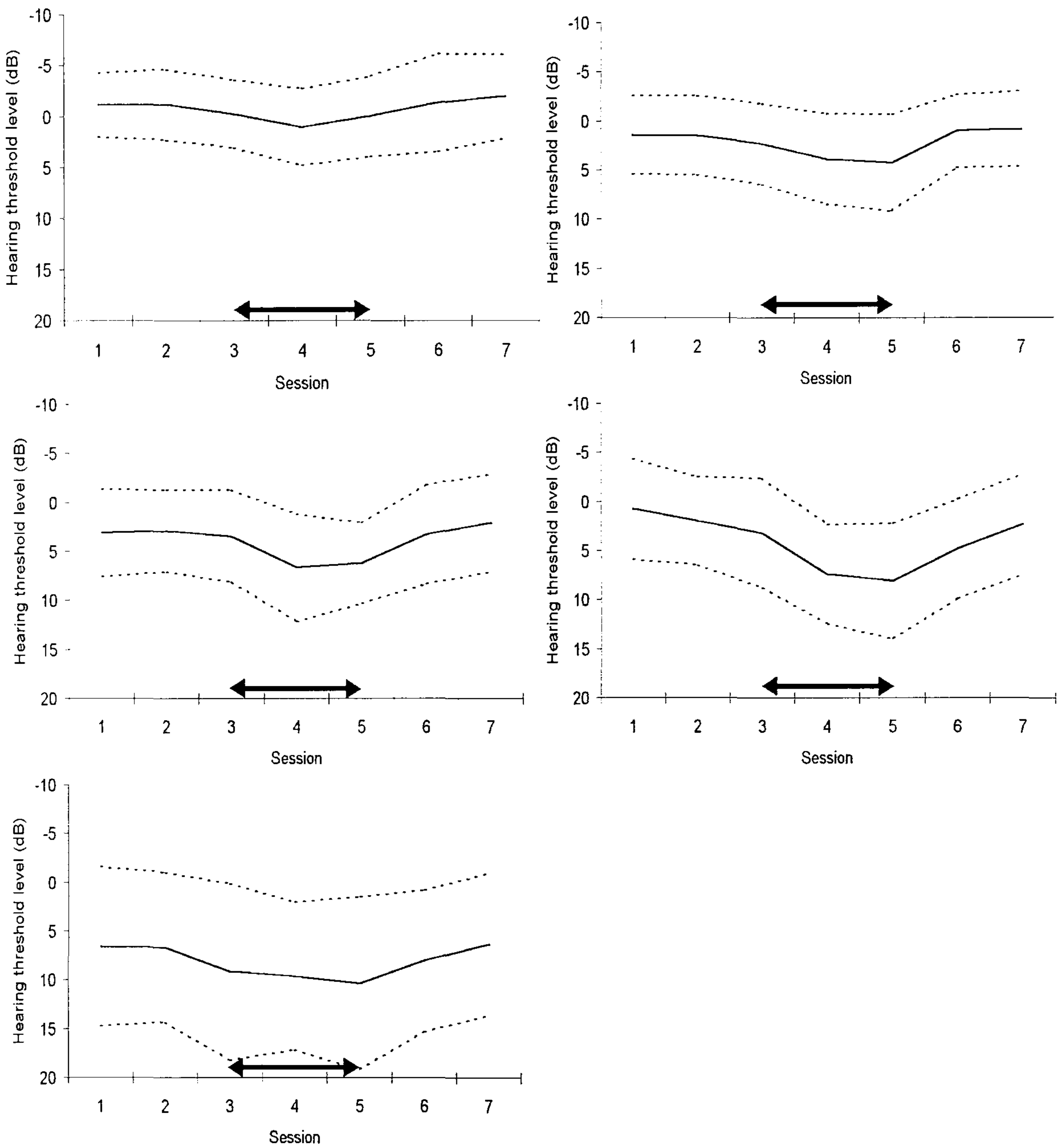
Figure 6-3: Plasma salicylate concentration values for each of the experimental subjects. Key. grey shading: session 3, black shading: session 4, white shading: session 5.

6.6.3 Hearing threshold level

Changes in hearing threshold with aspirin were measured. Mean aspirin-induced hearing threshold shifts over the seven-day period are shown in Figure 6-4 (data from left and right ears were averaged).

Figure 6-4 shows at each frequency an overall trend of increasing HTL with aspirin consumption. The highest mean change was measured at sessions 4 and 5, and this effect was greatest at the higher frequencies. To assess whether the changes in HTL with aspirin consumption were significant, a repeated measures ANOVA was performed. A statistical contrast was used that compared the mean of each session to the mean of the pre-aspirin sessions (a 'simple' contrast). For these data, thresholds at sessions 3, 4, 5, 6 and 7 were compared with the mean of sessions 1 and 2 for significant differences. This showed a significant effect of aspirin on HTL at frequencies of 1 kHz in the left ear only and at 2, 3, 4 and 6 kHz in both the left and right ears. At most frequencies, this effect was only significant at sessions 4 and 5. The most clearly significant shift was at 4 kHz.

Repeated measures ANOVA, using a differences contrast was used to analyse significant differences in threshold shifts between the left and right ears. This showed no difference in the effect of aspirin on the left and right ears. Table 6-4 shows the maximum HTL shifts experienced by each subject in each ear over sessions 3, 4 or 5. Aspirin had a variable effect on HTL amongst subjects, generating a range of aspirin-induced hearing threshold shifts. The maximum shift elicited was 21.5 dB at 6 kHz. The changes in hearing were in general smaller than expected, which meant that even with an aspirin-induced hearing loss most subjects' HTL were still within normal limits (better than 20 dB HL). None of the subjects reached HTL that could be classed as a mild hearing loss; this was limited by the dosage of aspirin that was ethically acceptable.


Hearing threshold level (dB)
-10
-5
0
5
10
15
20
1
2
3
4
5
6
7
Session

Appendix 10 shows the individual threshold shifts for each ear. Many subjects only showed a threshold shift at a single frequency in one or both ears. However there was a small group of susceptible subjects that showed consistently increased thresholds in response to salicylate at all or most of the frequencies tested. For the purposes of this experiment, susceptible subjects were defined as those who showed threshold shifts of greater than 5 dB at three or more frequencies in the same session, in at least one ear. Four subjects (subject numbers 7, 9, 10 and 13) fitted this definition and Figure 6-5 shows the course of the TTS of each member of the susceptible group.

Table 6-4: Maximum aspirin-induced hearing threshold shifts (dB) in sessions 3, 4 or 5

Subject number	Ear	Frequency (kHz)				
		1	2	3	4	6
1	L	−1.36	0.3	0.8	1.6	0.2
	R	7.0	4.9	6.4	**19.0**	3.8
2	L	0.7	3.1	2.8	**16.9**	-1.5
	R	3.2	1.0	3.8	6.9	5.4
3	L	3.5	5.2	2.1	3.4	**10.5**
	R	2.8	-0.5	5.4	8.8	**13.8**
4	L	5.3	−0.7	4.9	8.5	5.9
	R	3.9	−0.5	8.2	3.8	8.2
6	L	4.1	2.9	2.4	7.4	4.2
	R	−0.3	2.8	1.2	2.5	**21.5**
7	L	4.8	7.7	9.4	8.1	**18.3**
	R	**14.4**	9.5	8.4	**18.4**	**17.1**
8	L	2.2	5.1	5.5	−0.4	4.5
	R	0.2	3.4	7.7	6.6	8.7
9	L	6.6	5.3	9.0	**13.3**	7.2
	R	3.3	1.7	4.7	**16.7**	0.8
10	L	5.3	9.4	7.6	8.3	**12.1**
	R	7.1	8.3	4.9	**16.9**	8.6
11	L	2.0	7.1	7.3	2.5	6.4
	R	−0.2	2.8	4.8	3.4	4.2
12	L	3.9	3	5.5	7.2	7.4
	R	2.8	6.9	3.7	6.1	9.6
13	L	6.2	4.3	**14.0**	6.8	**21.5**
	R	7.9	4.7	3.3	5.1	9.0
14	L	1.1	5.9	3.9	9.0	−0.4
	R	0.3	5.4	7.2	4.1	3.6
15	L	5.5	6.2	5.9	9.9	2.7
	R	3.7	4.5	**14.7**	7.2	3.9
16	L	−1.5	4.0	3.3	8.4	9.8
	R	−0.5	−0.8	4.8	4.2	9.4
17	L	2.4	3.0	4.5	1.0	−0.5
	R	5.0	4.4	5.4	7.6	−7.0
19	L	1.3	4.3	4.0	1.8	4.4
	R	−0.5	7.7	4.0	**13.4**	7.6

Changes greater than 10 dB are shown in bold type.

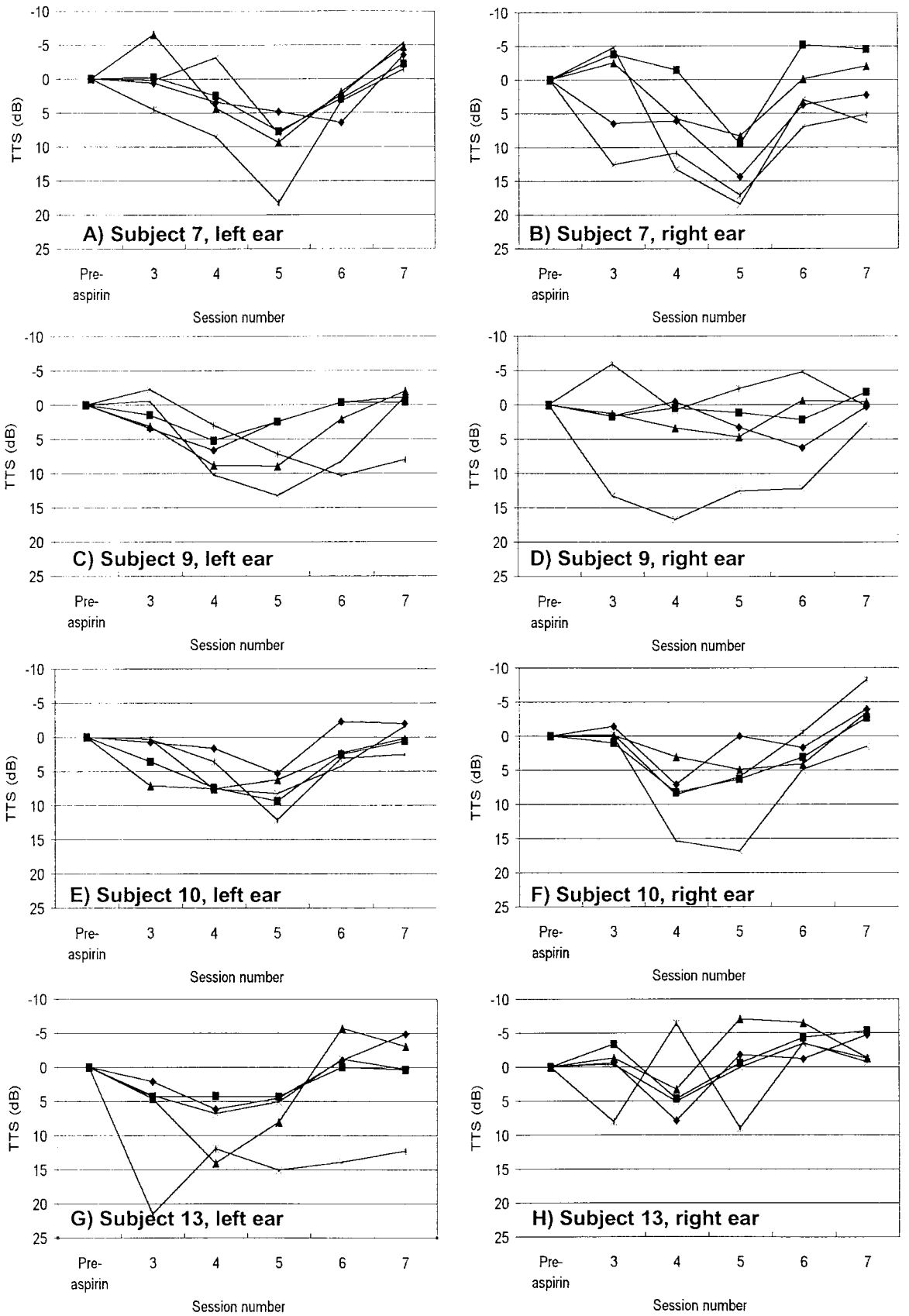


Figure 6-5: Temporary hearing threshold shift (TTS) results of susceptible subjects. Key to symbols - diamonds: 1 kHz, squares: 2 kHz, triangles: 3 kHz, crosses: 4 kHz, stars: 6 kHz.

Whereas Figure 6-5 shows four subjects with consistent changes in pure tone threshold on aspirin consumption, other subjects showed only a small effect of aspirin on hearing.

The relationship between plasma salicylate concentration and HTL shifts was also examined. It was expected that the subjects with the greatest, consistent HTL shifts were those with the highest plasma salicylate concentration. Figure 6-6 plots graphically the plasma salicylate against the shift in mean HTL at 1, 2 and 3 kHz, and also against the mean HTL at 3, 4 and 6 kHz. This shows wide variation between subjects and only a weak trend of an increase in HTL shift with increasing plasma salicylate concentration. HTL shift is therefore not explained by plasma salicylate concentration.

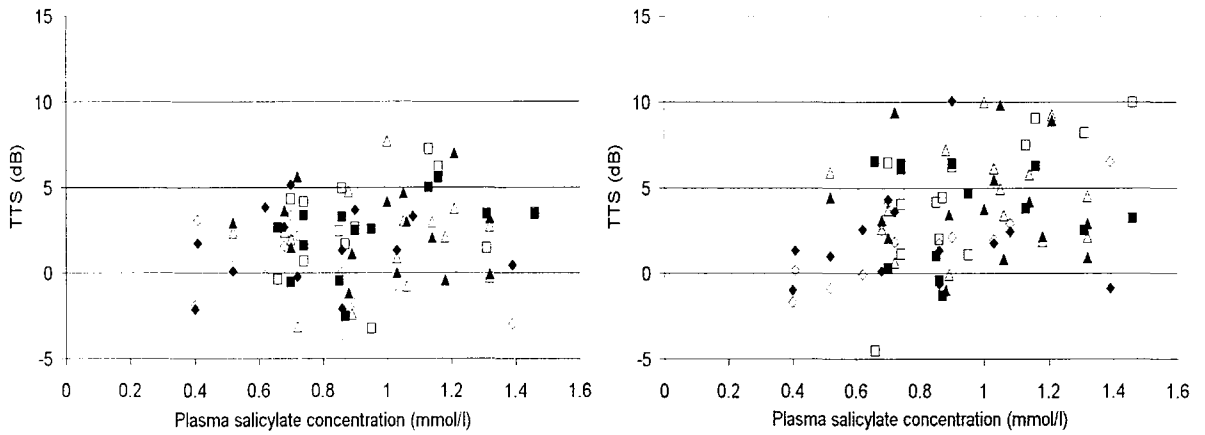


Figure 6-6: Plasma salicylate concentration plotted against temporary threshold shift (TTS) (dB). A) HTL: mean 1, 2 and 3 kHz B) HTL: mean 3, 4 and 6 kHz. Key to symbols – diamonds: session 3, squares: session 4, triangles: session 5. Filled symbols: left ear, open symbols: right ear.

6.6.4 SOAE

The frequency spectrum of the SOAE recordings from sessions 1 and 2 were analysed visually to identify repeatable peaks greater than 3 dB above the noise floor. Five out of the seventeen subjects (30%), and eight out of thirty four ears (24%) had repeatable SOAE, as summarised in Table 6-5. 29% of the SOAE were recorded in the left ear, and 71% in the right ear. All SOAE were recorded between 1 to 2 kHz.

The changes in SOAE with aspirin consumption are shown in Figure 6-7. This showed a reduction in the amplitude of SOAE with aspirin consumption. For most subjects the SOAE was not recordable above the noise floor during aspirin consumption (subjects 1, 3, 4 and 9), whereas for subject 14 the amplitude of the SOAE reduced markedly but was still recordable.

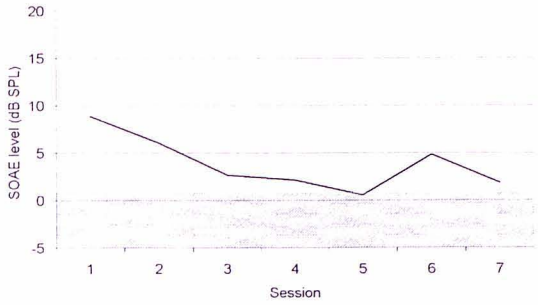
Table 6-5: Summary of subjects with SOAE (>3 dB SNR), showing the ear and frequency of SOAE

Subject number	Left ear	Right ear
1		1140 Hz
3	1856 Hz	2240 Hz
4		1904 Hz
9		1328 Hz
16	1328 Hz	1488 Hz

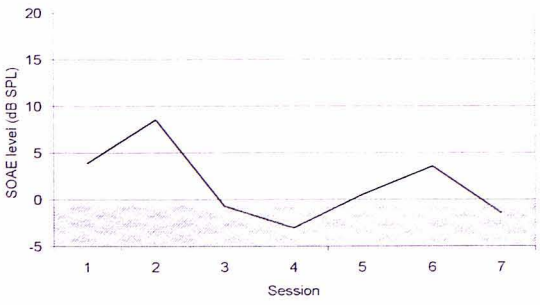
6.6.4.1 SOAE and HTL

The relationship between SOAE and HTL was examined in all subjects and ears. A correlation coefficient analysis was performed between SOAE and the HTL frequencies in the same ear, across the seven sessions.

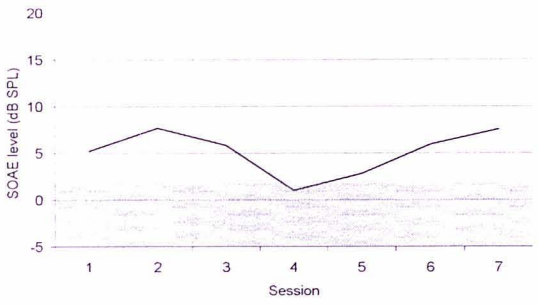
Table 6-6 summarises the significant relationships between SOAE and HTL. This showed a significant relationship between SOAE and HTL in five out of seven ears (71%), and four out of five subjects (80%). In two of the ears examined, the SOAE was significant correlated with HTL at a frequency close to the frequency of the SOAE. However in three of the ears, the SOAE was significantly correlated to HTL several octaves higher. Figure 6-8 gives an example of the relationship between SOAE and HTL in subject 1.



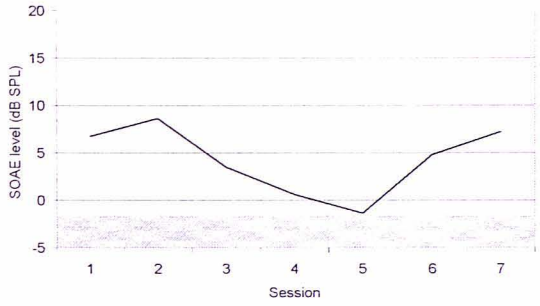
A) Subject 3



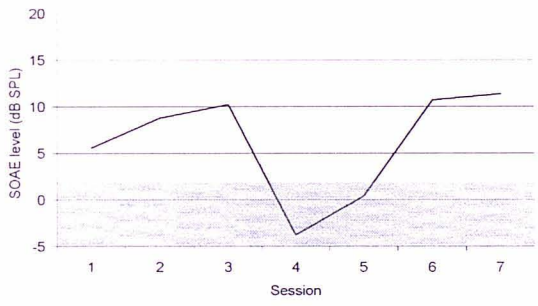
B) Subject 3



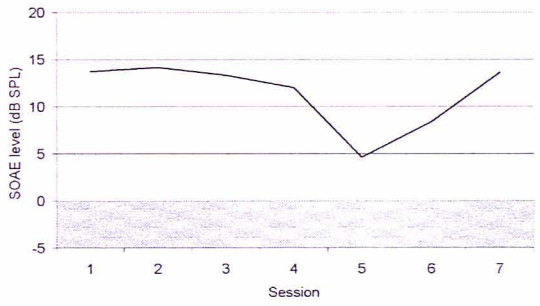
C) Subject 1



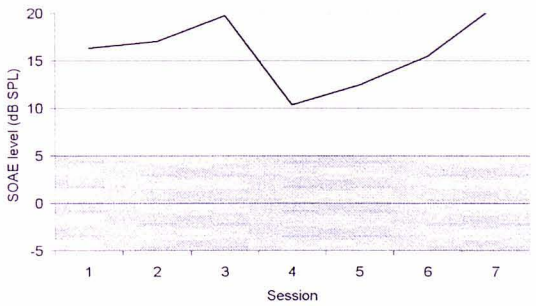
D) Subject 4



E) Subject 9



F) Subject 16



G) Subject 16

Figure 6-7: Changes in SOAE over time. A) Subject 3, left ear. B) Subject 3, right ear. C) Subject 1, right ear. D) Subject 4, right ear. E) Subject 9,right ear. F) Subject 16,right ear. G) Subject 16,left ear. Mean noise floor (averaged over the seven sessions) shown by shaded area.

Table 6-6: Correlation coefficients of the significant relationships between SOAE and HTL

Subject number	Ear	SOAE frequency (Hz)	HTL frequency (kHz)				
			1	2	3	4	6
1	R	1140	-0.94 (P=0.001)	-0.93 (P=0.002)			
3	L	1856					
3	R	2240					
4	R	1904	-0.86 (P=0.01)				
9	R	1328			-0.78 (P=0.03)		
16	L	1328				-0.90 (P=0.005)	
16	R	1488					-0.75 (P=0.04)

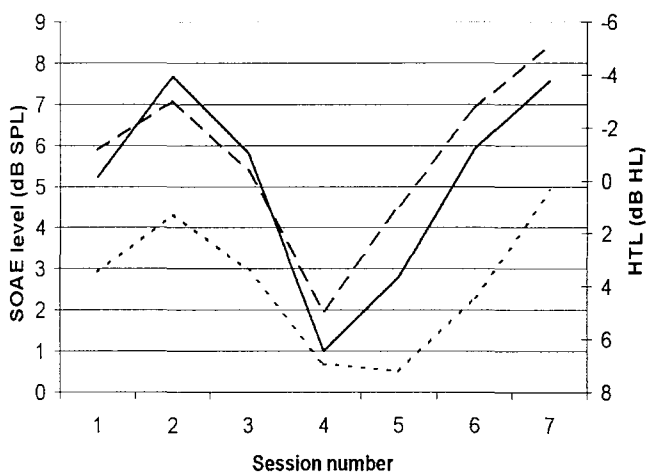


Figure 6-8: The relationship between SOAE and HTL in subject 1, right ear. This shows a very high correlation between the change in SOAE and the change in HTL Key: solid line: SOAE, dashed line: 1 kHz HTL, dotted line: 2 kHz HTL

6.6.5 DPOAE

6.6.5.1 DPOAE I/O functions

Changes in DPOAE level were examined. Figure 6-9 shows the mean DPOAE level shifts across sessions 1 to 7, plotted by f_2 stimulus level. Data combine both the left and right ears.

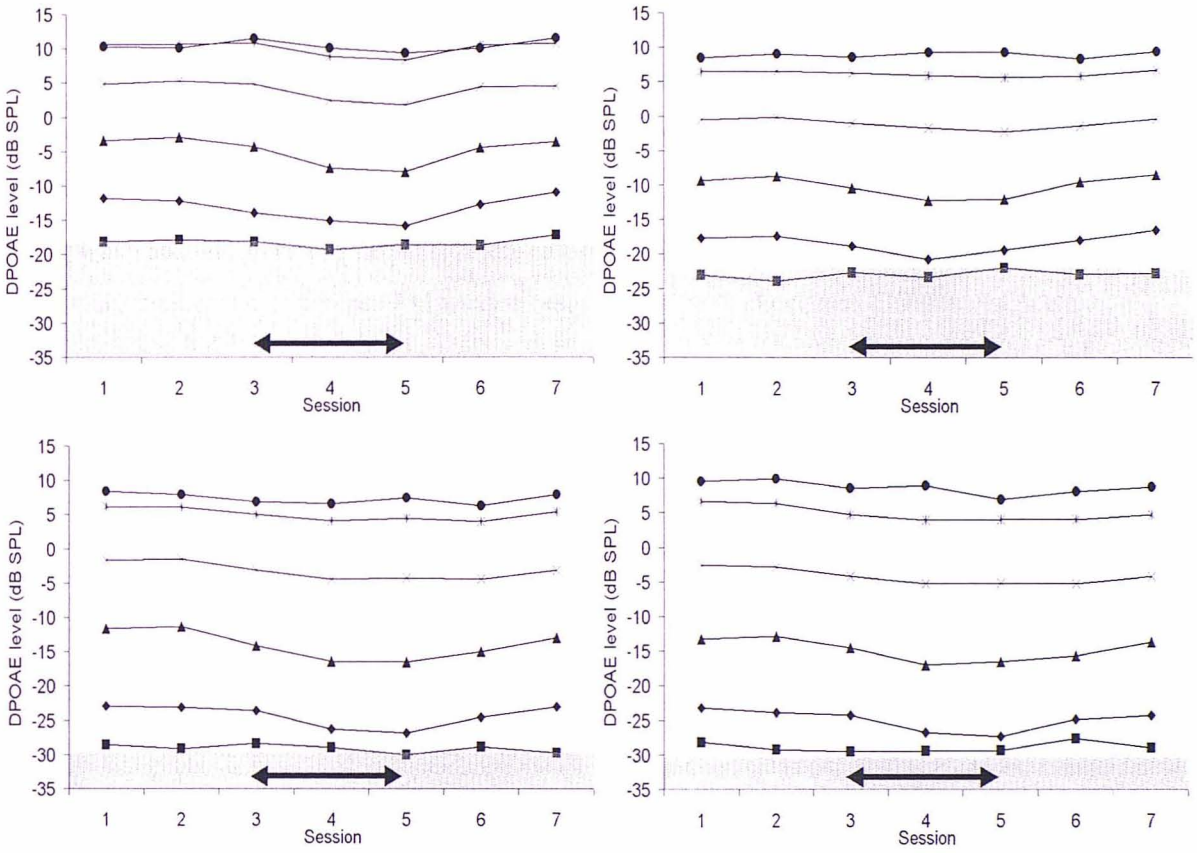


Figure 6-9: Mean change in DPOAE level at each test session (left and right ears combined). A) 2 kHz. B) 3 kHz. C) 4kHz. D) 6 kHz. Key to symbols – circles: L2 70 dB, asterisks: 60 dB, crosses: 50 dB, triangles: 40 dB, diamonds: 30 dB, squares: 20 dB. Arrow shows duration of aspirin consumption. Mean noise floor shown by shaded area.

Figure 6-9 shows a reduction in DPOAE level with salicylate, dependent on stimulus intensity. DPOAE evoked at lower stimulus levels were the most sensitive to salicylate, with the greatest reduction in level occurring at stimulus levels of 40 and 30 dB. The results at a stimulus level of 20 dB were around or below the noise floor. A repeated measures ANOVA was performed to assess the effect of the within-subject factors: session, frequency, stimulus level and ear on DPOAE level. This showed a significant effect of session ($P< 0.001$), frequency ($P <0.001$) and level ($P< 0.001$). There was no significant difference between the effect of salicylate on the left and right ears ($P>0.05$)

According to the framework an increase in I/O function slope is expected, along with a reduction in level with salicylate consumption. I/O functions were plotted at each frequency. Mean changes in the I/O function (calculated across subjects and ears) from pre-aspirin to session 5 are shown in Figure 6-10.

The mean I/O functions at each frequency showed similar changes with aspirin consumption. Each function showed the largest reduction in level at stimulus levels between 30 to 50 dB. Functions at 2, 3 and 4 kHz showed no mean change in DPOAE level at 70 dB stimulus level. At 6 kHz there was a mean change in level across the stimulus levels including a reduction at 70 dB. The differential reduction in level at the lower stimulus levels compared to the higher stimulus levels means that the I/O function showed a reduction in nonlinearity.

There was variation between subjects in the responses of the I/O functions to aspirin. Figure 6-11 gives examples of the different I/O function responses to salicylate from different subjects.

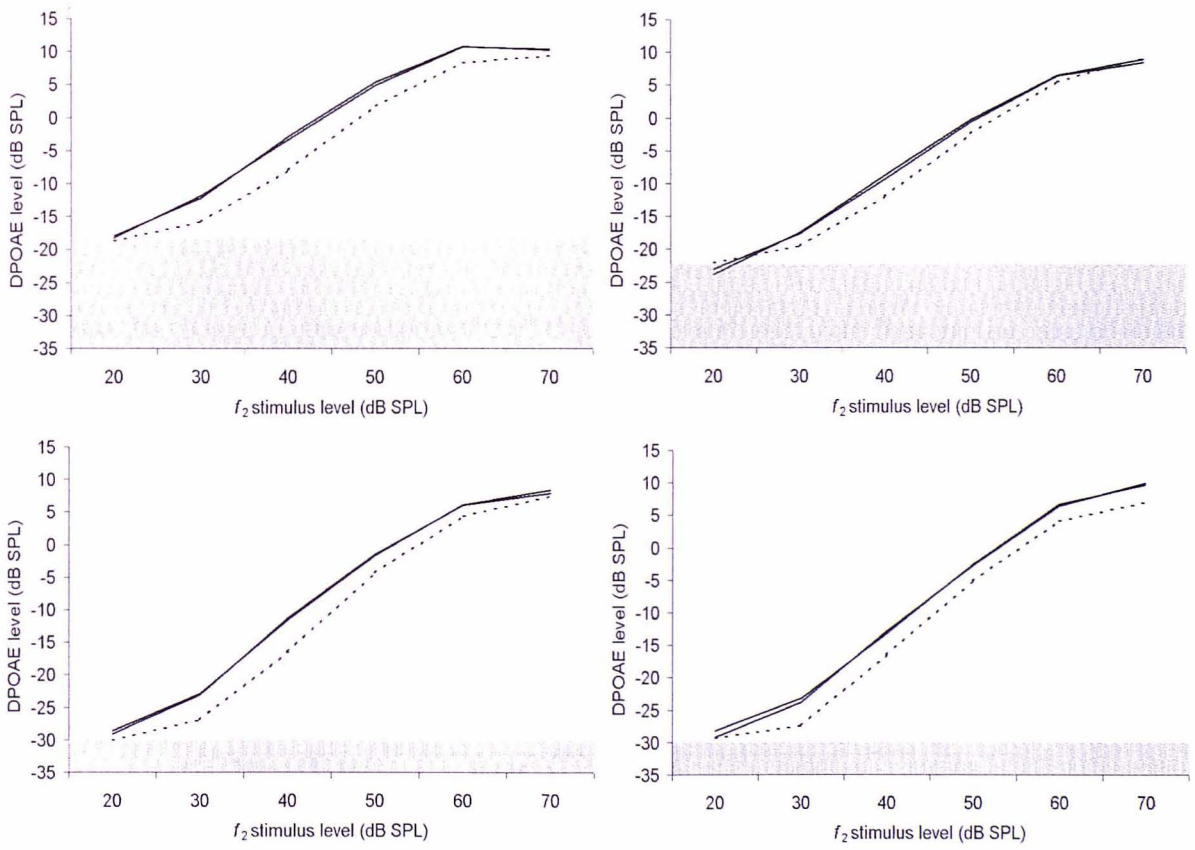


Figure 6-10: Mean DPOAE I/O functions (left and right ears combined). A) 2 kHz. B) 3 kHz. C) 4 kHz. D) 6 kHz. Key: dark solid lines: sessions 1 and 2, thin broken lines: session 5. Mean noise floor shown by shaded area.

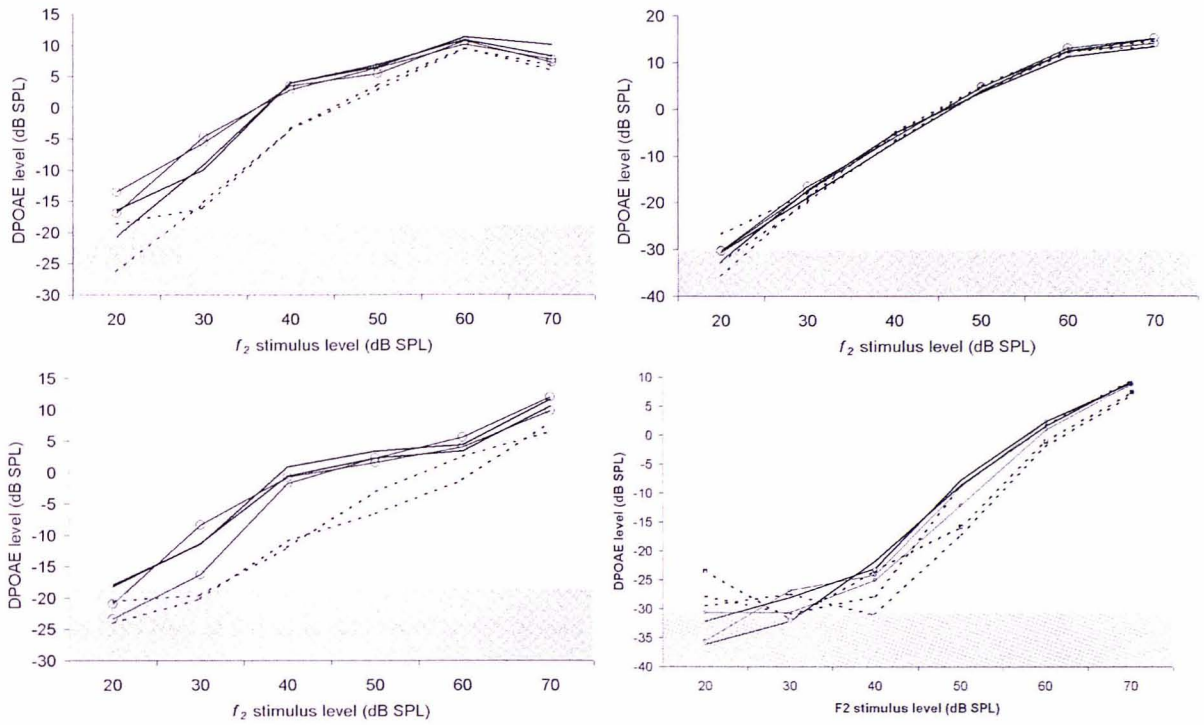


Figure 6-11: Examples of individual subject DPOAE I/O functions. A) Subject 8, right ear, 2 kHz. B) Subject 11, right ear, 6 kHz. C) Subject 12, right ear, 2 kHz. D) Subject 10, left ear, 4 kHz. Key – solid lines: sessions 1 and 2, dashed lines: session 3, 4 and 5, solid lines with circles: session 6 and 7. Mean noise floor shown by shaded area.

To assess the change in compression of the I/O functions with salicylate consumption, slopes of the functions were calculated between stimulus levels of 40 to 60 dB using linear regression. The results of the median slopes of the I/O function from sessions 1 and 2 and session 5 are shown in Table 6-7.

Table 6-7: Median slope values of DPOAE I/O functions pre-aspirin and at session 5 (dB/dB)

Session	Frequency (kHz)			
	2	3	4	6
Mean 1 & 2	0.69	0.76	0.89	0.93
5	0.84	0.85	1.05	1.02

The results show an increase in the slope of the I/O function of approximately 0.1 dB/dB as a result of salicylate consumption. Although the changes are small, this is consistent with the model of I/O functions. The increase in slope indicates a reduction in compression with salicylate.

Individual pre-aspirin DPOAE I/O functions were examined for the presence of a notch at mid-intensity stimulus levels. Analysis of all DPOAE I/O functions showed that none of the subjects had a notch in any I/O function at any frequency.

6.6.5.2 DPOAE level and HTL

Group analysis

Using individual I/O functions, the change in DPOAE level with aspirin consumption at each stimulus level was estimated for each subject. It was predicted that the changes in OAE level were related to HTL. Changes were calculated by comparing the mean I/O function from sessions 1 and 2 with that from session 5 for each subject. Results from session 5 were used as the DPOAE from this session were significantly different from the pre-salicylate sessions across the frequency range. Changes in DPOAE level were examined at stimulus levels of 30, 40, 50 and 60 dB. These stimulus levels were used as DPOAE at these levels were most sensitive to salicylate.

Changes in DPOAE level with aspirin consumption at these stimulus levels were in the range +13 to –29 dB. To assess whether the group changes in DPOAE level were significantly correlated with the changes in HTL, a correlation analysis was performed. All subject data were combined and the change in DPOAE level was correlated with the change in HTL at each frequency. Significant results are shown in Table 6-8.

Table 6-8: Results of the correlation analysis relating the change in DPOAE level (independent variable) and the change in HTL (dependent variable)

DPOAE frequency (kHz)	HTL frequency (kHz)	f_2 level (dB)	Correlation coefficient	Slope	R-square value
3 average	1	50	–0.54**	–0.35	0.29
	1	60	–0.49**	–0.53	0.24
	3	50	–0.43*	–0.52	0.18
4 average	1	50	–0.42*	–0.30	0.17

Of the variables examined, there were only four significant relationships, of which the maximum correlation was 0.54 with an R-square value of 0.29, indicating a weak relationship and that the change in DPOAE explains only 30% of the variance in the change in HTL. Three out of the four

relationships showed DPOAE significantly correlated to HTL at 1 kHz. These results in general indicate a low correlation between the group changes in DPOAE and changes in HTL.

Graphical examples of significant relationships between the change in DPOAE level and the change in HTL are shown in Figure 6-12.

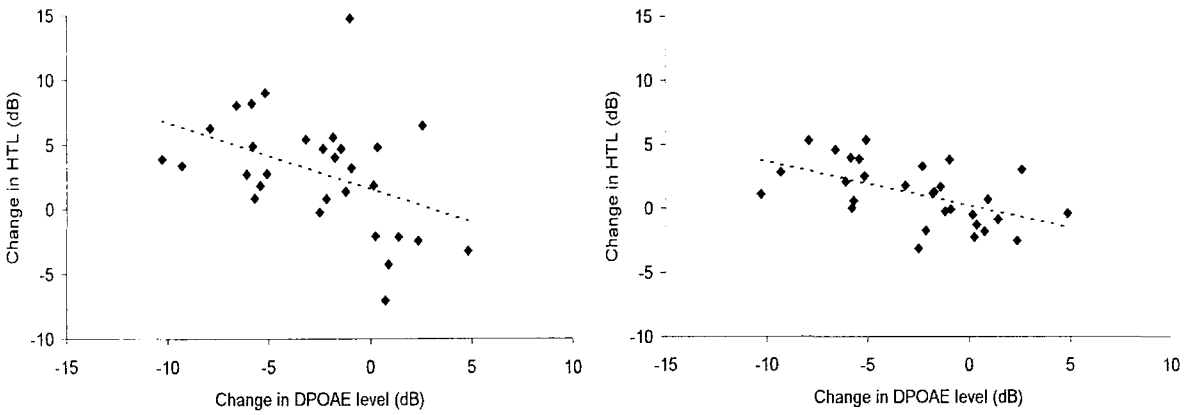


Figure 6-12: A) Change in DPOAE level (3 kHz, L2 50 dB) against the change in HTL at 3 kHz. B) Change in DPOAE level (3 KHz, L2 50 dB) against the change in HTL at 1 kHz. Linear regression line plotted.

Figure 6-12 shows a general trend of a reduction in DPOAE level associated with an increase in HTL. There was wide variability in the data, with some subjects showing changes in DPOAE level accompanied by little or no change in HTL. Both figures show that the relationship between the change in DPOAE level with HTL is not 1 dB/dB. In these examples a 1 dB reduction in DPOAE level is associated with a 0.5 and 0.3 dB reduction in HTL, dependent on stimulus level. DPOAE evoked by lower intensity level stimuli are more sensitive to changes than DPOAE evoked by higher-level stimuli, and therefore the slope of the relationship between DPOAE and HTL will be lower when DPOAE is evoked by lower stimulus levels.

Individual subject analysis

The relationship between DPOAE level and HTL was then analysed within each individual subject over the seven sessions. Correlation analysis was used to examine the relationship between HTL and DPOAE for each subject, ear, frequency and level.

In contrast to the results of the group analysis, the individual subject analysis gave highly significant results. Most subjects showed highly significant correlations between DPOAE level and HTL in both left and right ears for varying combinations of variables. There were different associations between the ears of individual subjects. Changes in DPOAE level and HTL with salicylate consumption were very closely related within ears. Ears with significant relationships had correlation coefficient values

of 0.7 and greater, indicating a very close association between the two variables. Most ears showed highly significant correlations between HTL and at least one DPOAE variable. Significant correlations were observed across the stimulus level range with L2 between 30 to 70 dB SPL. Figure 6-13 shows four examples of the high correlations between DPOAE and HTL.

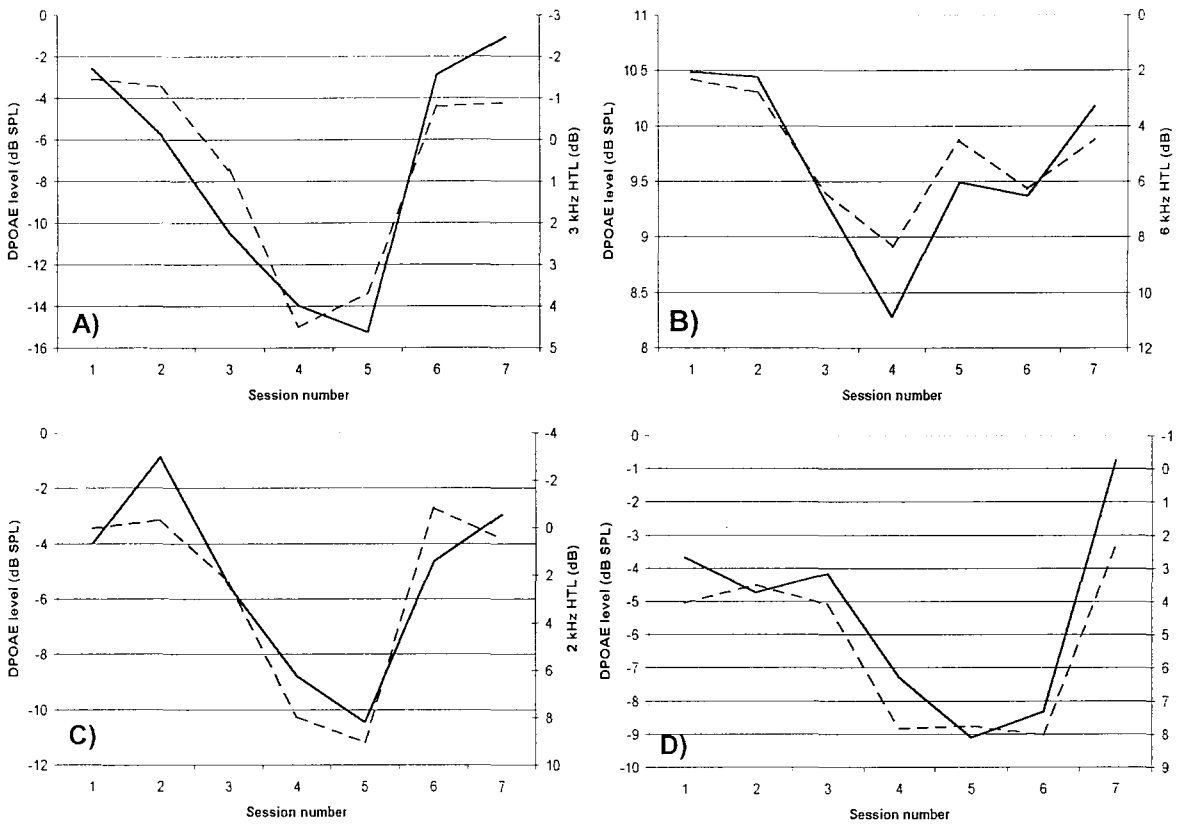


Figure 6-13: Association between DPOAE level and HTL with salicylate consumption. The scale on the axes was altered for each subject to illustrate the high correlation. A) Subject 8, left ear (DPOAE 2 kHz, L2 30 dB). B) Subject 8, right ear (DPOAE 6 kHz, L2 60 dB). C) Subject 10, left ear (DPOAE 3 kHz, L2 50 dB). D) Subject 10, right ear (DPOAE 4 kHz, L2 50 dB). Key – solid line: HTL, dashed line: DPOAE level. All DPOAE measures were greater than the noise floor.

The examples used illustrate that the change in DPOAE and HTL occurred at the same point in time and that the DPOAE changes did not precede the change in HTL. Each of the individual ears were examined to determine whether the change in DPOAE level preceded the change in HTL. Graphs of each significant correlation of the change in DPOAE and HTL were examined visually. The correlations were counted where there was a change in DPOAE without a change in HTL. This showed that most changes were simultaneous and the changes in DPOAE preceded the changes in HTL in only 4% of all correlations.

The number of significant correlations between DPOAE and HTL frequencies within subjects and ears was calculated. Five different HTL frequencies were correlated with four different DPOAE frequencies, and the number of significant associations between DPOAE and HTL frequencies for each ear was calculated. For each DPOAE/HTL frequency combination, a significant association was deemed present if there were significant correlations measured at more than one stimulus level for the DPOAE frequency in question. As a large number of variables were correlated, this approach was taken to ensure that only genuine relationships between DPOAE and HTL were counted, and correlations that were significant through chance were excluded (although it is possible that some genuinely significant correlations were excluded using this approach).

Table 6-9 summarises the number of significant relationships between DPOAE and HTL for each subject and ear. This shows a range of significant correlations across subjects and ears. There were differences between ears within subjects of the number of correlations. Figure 6-14 compares the number of significant correlations across ears. A high number of correlations in one ear do not predict a high number in the contralateral ear.

Table 6-9: Summary of the number of significant correlations (P =0.05) between HTL and DPOAE level variables (out of a maximum of 20 per ear)

Subject number	Left ear (max 20)	Right ear (max 20)	Left/right total (max 40)
1	0	1	1
2	3	0	3
3	0	1	1
4	4	2	6
6	0	0	0
7	7	3	10
8	8	4	12
9	9	4	13
10	10	16	26
11	2	1	3
12	2	2	4
13	7	0	7
14	1	7	8
15	1	0	1
16	4	1	5
17	8	6	14
19	1	2	3
25^{th} percentile	1	1	1.5
Median	3	2	4.5
75^{th} percentile	7	4	9.5

Figure 6-15 summarises the cumulative percentage of ears and subjects with significant correlations. There was a maximum of 20 possible DPOAE/HTL frequency combinations: therefore using a significance level of 0.05, 1/20 of these were likely to be due to chance. Approximately 60% of ears and 70% of subjects had two or more significant correlations between DPOAE and HTL. The data were examined to determine which DPOAE and HTL frequencies were most likely to be significantly related.

Figure 6-16 summarises the number of significant correlations between the different variables for all ears. This showed the highest number of significant relationships between DPOAE at 4 kHz and HTL at 3 kHz. The HTL frequency of 6 kHz showed the smallest number of significant relationships.

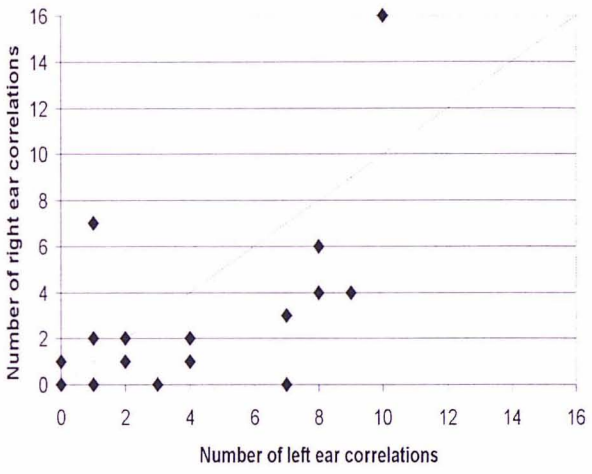


Figure 6-14: Relationship within subjects between the number of significant correlations (DPOAE/HTL) in the left and right ears. The dashed line shows a 1:1 relationship. There was a 57% correlation between the number of correlations in the left and right ears.

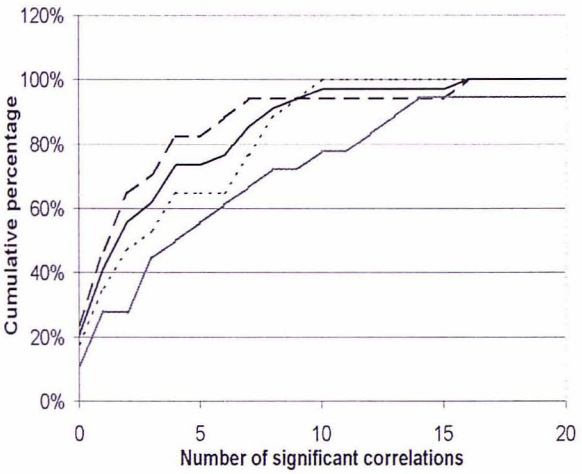


Figure 6-15: Cumulative % of ears/subjects with significant correlations. Key – dark solid line: left/right ears combined, dashed line: right ear, dotted line: left ear, grey solid line: subjects.

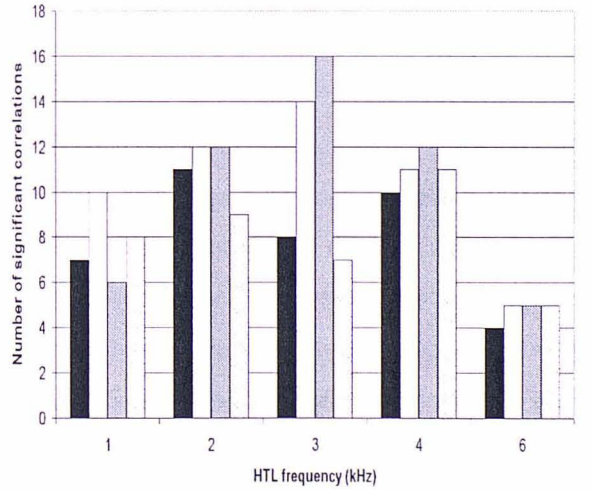


Figure 6-16: Summary of the number of significant correlations between HTL and DPOAE frequencies during salicylate consumption. The most common association was between HTL at 3 kHz and DPOAE at 4 kHz. Key – DPOAE frequency black bars: 2 kHz, white bars: 3 kHz, grey bars: 4 kHz, stippled bars: 6 kHz.

The data were analysed further to determine factors that predicted which ears showed significant associations between DPOAE and HTL. Possible factors that were considered important were:

- Size of the change in DPOAE level
- Size of the change in HTL
- Initial HTL level

The correlation coefficients relating DPOAE level and HTL were plotted against each of the possible predictors. Where the correlation coefficient was significant, the coefficient value was used; where the relationship was insignificant, a value of zero was used. If correlation coefficient was related to the predictor variable, the data would cluster into two groups: significant relationships at one end of the graph and insignificant relationships at the other end. Each of the predictor variables was examined, but there was no marked effect of these variables on the correlation coefficient and there was wide variation across subjects.

Although no predictor was identified, it seems likely that the size of change in HTL is important. The susceptible subjects (those subjects who showed consistently raised thresholds with salicylate) all showed a large number of correlations between HTL and DPOAE across the frequency range for both ears.

In those ears with significant relationships between DPOAE and HTL, the individual relationships between the variables were examined in further detail. Figure 6-17 and Figure 6-18 illustrate the relationship between the change in HTL and the change in DPOAE for several ears. This shows that although each ear had a different initial DPOAE level, the slope of the change relating DPOAE and HTL was similar in each subject.

It was therefore proposed that if the slope values relating DPOAE and HTL are similar between ears, then it may be possible to predict the extent of the change in HTL purely from the change in DPOAE level as long as the initial starting levels are known. The linear regression slope relating HTL and DPOAE was calculated for each significant relationship for each subject and ear. DPOAE was used as the independent variable.

The median slope was calculated for the relationship between HTL and DPOAE at the different stimulus levels. These are summarised in Figure 6-19 at each frequency. In general, the lower slope values were associated with the lower stimulus levels as expected (because low intensity levels more sensitive) and higher slope values with the higher stimulus levels. There was some variation in slope values both between subjects and also within subjects at different frequencies, however the variation was fairly small. The variability in slope was greatest at the lower stimulus levels.

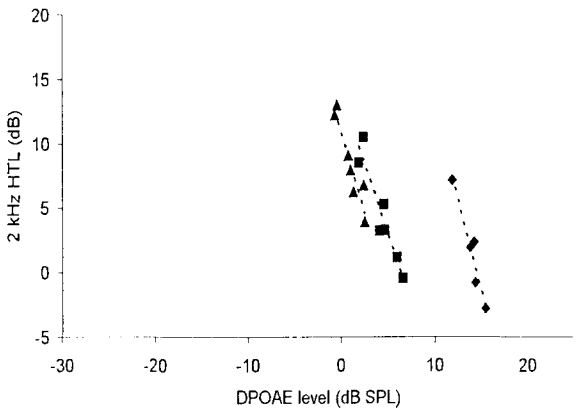


Figure 6-17: 2 kHz DPOAE level (L2 60 dB) versus 2 kHz HTL across sessions. Each subject shows a similar relationship between the change in DPOAE and the change in HTL. R-square values ranged from 0.88 - 0.92. Key to symbols – diamonds: subject 7, squares: subject 10, triangles: subject 19.

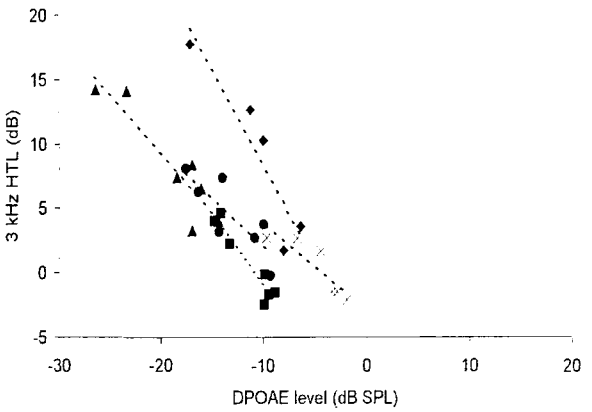


Figure 6-18: 4 kHz DPOAE level (L2 40 dB) versus 3 kHz HTL across sessions. Most subjects show a similar relationship between the change in DPOAE and the change in HTL. R-square values ranged from 0.7 - 0.9. Key to symbols – diamonds: subject 7, squares: subject 8, triangles: subject 9, circles: subject 10, crosses: subject 13.

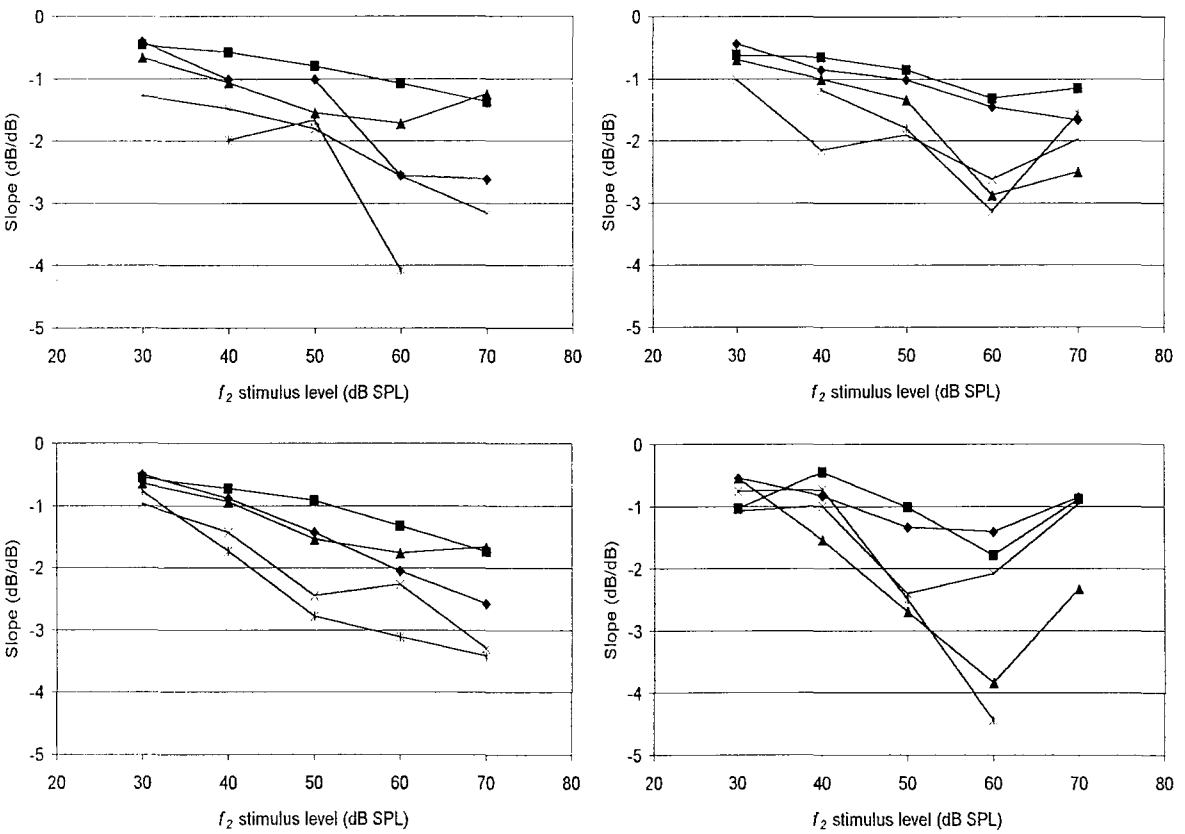


Figure 6-19: Median slope values of the relationship between DPOAE (independent variable) and HTL (dependent variable). Graphs are plotted at each DPOAE frequency. Key to symbols – HTL frequencies squares: 1 kHz, diamonds: 2 kHz, triangles: 3 kHz, crosses: 4 kHz, stars: 6 kHz.

6.6.5.3 DPOAE and SOAE

The DPOAE from ears with measurable SOAE were examined in more detail. Several questions were investigated. Firstly did ears with SOAE have larger amplitude DPOAE than ears with absent SOAE, and did they change differently with aspirin consumption?

The group was split into two: the SOAE positive group (SOAE+) contained all ears with measurable SOAE and the SOAE negative group (SOAE–) contained all ears with no measurable SOAE. Table 6-10 compares the mean pre-aspirin DPOAE levels from sessions 1 and 2 in the SOAE+ and the SOAE – groups. The SOAE+ group had larger DPOAE levels than the SOAE– group. The difference was marked at the lower frequencies, close to the frequency of the SOAE. An independent samples t-test was used to test for significant differences. This showed borderline significant differences at 2 and 3 kHz, and no significant differences at 4 and 6 kHz.

Table 6-10 also compares the changes in DPOAE during aspirin consumption between the two groups and Figure 6-20 shows the DPOAE I/O functions pre- and peri-aspirin for the two groups. This showed a trend in the SOAE+ group for changes in DPOAE to be larger at the lower stimulus levels than in the SOAE– group. An independent samples t-test was used to test for significance, and this showed that this trend was not significant. There was a significant difference at 2 kHz, with the SOAE– group showing significantly greater changes in DPOAE than the SOAE + ears.

Also investigated was whether ears with SOAE more likely to have significant relationships between the change in DPOAE and HTL than ears with absent SOAE. As the data were not normally distributed, a Mann-Whitney U test was used to compare the number of significant correlations between the two SOAE groups. This showed no significant difference in the number of significant correlations between the SOAE+ and SOAE– groups (P=0.379).

Table 6-10: Comparison of the absolute DPOAE levels, and changes in DPOAE in the SOAE+ and SOAE– groups

Frequency (kHz)	F2 stimulus level (dB SPL)	Mean DPOAE level sessions 1/2 (dB)		Significance value (P)	Mean change in DPOAE level from sessions 1/2 to session 5 (dB)		Significance value (P)
		SOAE+	SOAE –		SOAE+	SOAE –	
2 kHz	30	-4.786	-14.227	0.09	-4.54	-3.52	0.67
	40	1.818	-4.630	0.16	-1.692	-5.769	0.03*
3 kHz	30	-12.173	-17.886	0.07	-3.87	-2.20	0.39
	40	-4.120	-9.528	0.05	-2.706	-3.718	0.32
4 kHz	30	-20.185	-22.764	0.38	-6.263	-2.436	0.10
	40	-8.914	-11.281	0.32	-5.231	-5.023	0.91
6 kHz	30	-21.364	-23.360	0.45	-5.731	-3.828	0.35
	40	-10.687	-12.153	0.48	-4.702	-5.418	0.71

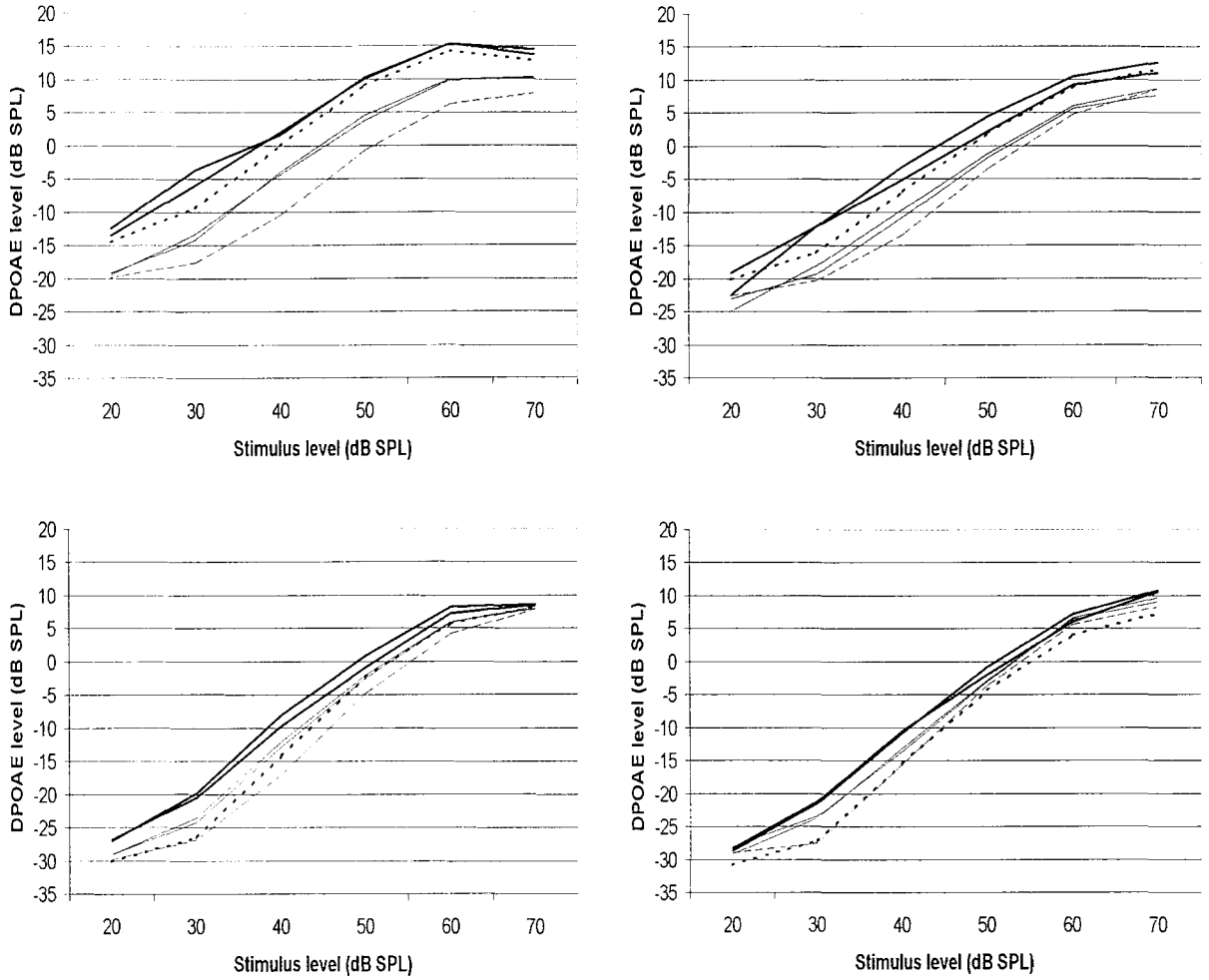


Figure 6-20: A comparison of DPOAE I/O functions from SOAE+ and SOAE– groups. A) 2 kHz. B) 3 kHz. C) 4 kHz. D) 6 kHz. On average the DPOAE from the SOAE+ group had higher amplitude levels than DPOAE from the SOAE– group, however this was not significantly different. Key – thick dark lines: SOAE+ group, thin lines: SOAE– group, solid lines: pre-aspirin, sessions 1 and 2, dashed lines: peri-aspirin, session 5.

6.6.6 TEOAE

6.6.6.1 TEOAE I/O functions

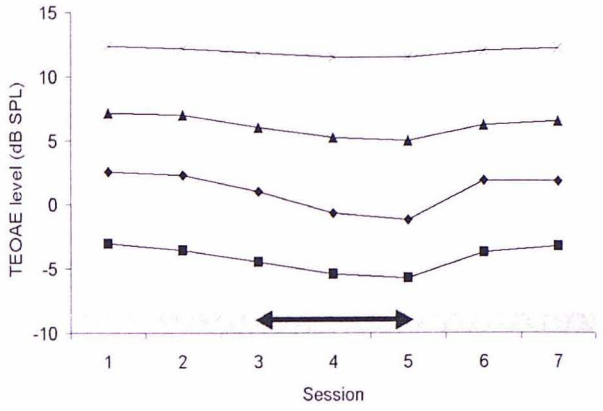
TEOAE at each click level were filtered into 1/6-octave bands and TEOAE level was calculated for each frequency band, and also for the broadband response. Changes in TEOAE level were examined.

Figure 6-21 shows the mean TEOAE level shifts across sessions 1 to 7, plotted by click level. Responses at 90 dB were not included. Data combine both left and right ears. Figure 6-21shows a reduction in TEOAE level with salicylate, dependent on stimulus level. TEOAE evoked by 80 dB clicks were relatively insensitive to salicylate, and showed little or no change in level. TEOAE evoked by lower click levels were more sensitive to salicylate, with TEOAE evoked by clicks of 70 and 60 dB showing the greatest reduction in level. The noise floor of the equipment, which was approximately −10 dB, was the limiting factor for recording responses to click levels at 50 dB or below. This was also a problem at 60 dB to a lesser extent.

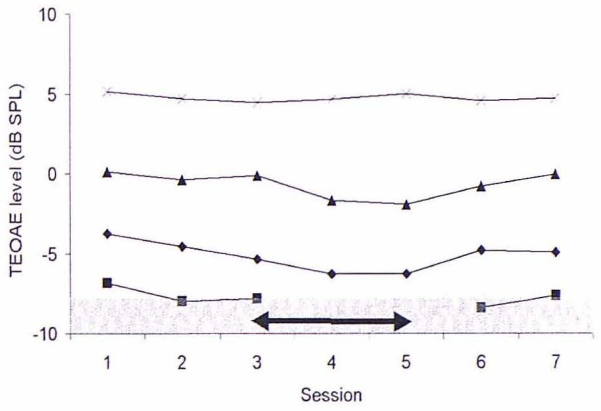
It was not possible to perform a repeated measures ANOVA to assess the effect of ear, frequency, level and session on TEOAE. No data were available for TEOAE responses below the noise floor, and for this reason there were large amounts of missing data particularly during salicylate consumption. The statistical test of repeated measures ANOVA is not able to cope with missing data, and for this reason it could not be used.

According to the framework, salicylate consumptions is expected to result in an increase in I/O function slope along with a reduction in level. I/O functions were plotted at each frequency. Mean changes in the I/O function (calculated across subjects and ears) from pre-aspirin to session 5 are shown in Figure 6-22.

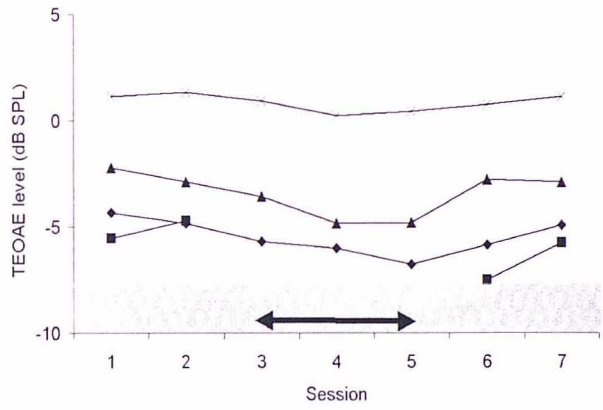
The I/O functions at the different frequencies showed slight differences in the effect of salicylate. Functions at 1 and 2 kHz and the broadband response showed similar changes to the DPOAE I/O functions with the largest reduction in level at the lower stimulus levels. This was consistent with the framework. The functions at 3 kHz showed a general reduction in level across the stimulus levels, giving a downward shift of the function. There was variation between subjects and ears in the responses of the I/O functions to aspirin. Figure 6-23 gives examples of the different I/O function responses to salicylate from different subjects.



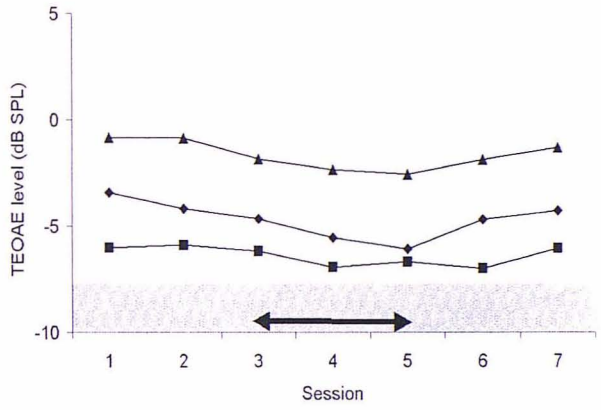
A) Broadband



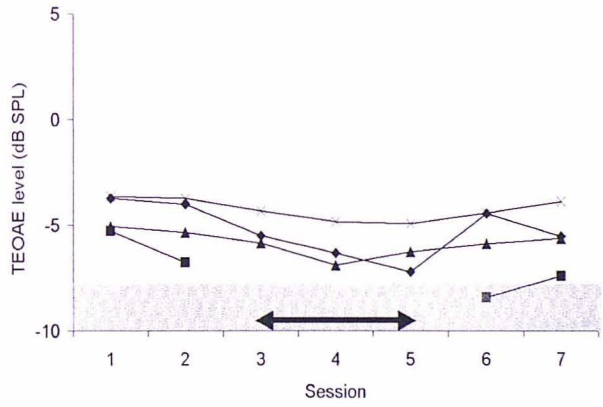
B) 1 kHz



C) 2 kHz

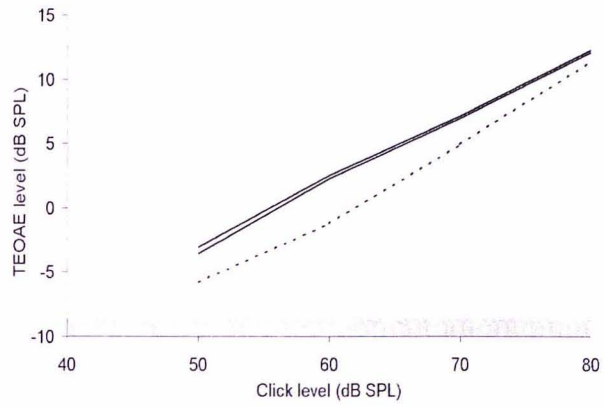


D) 3 kHz

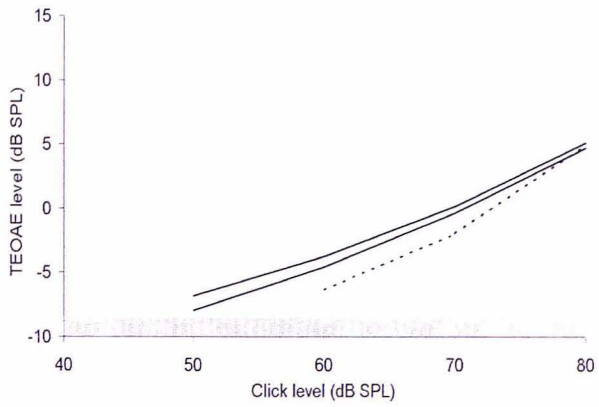


E) 4 kHz

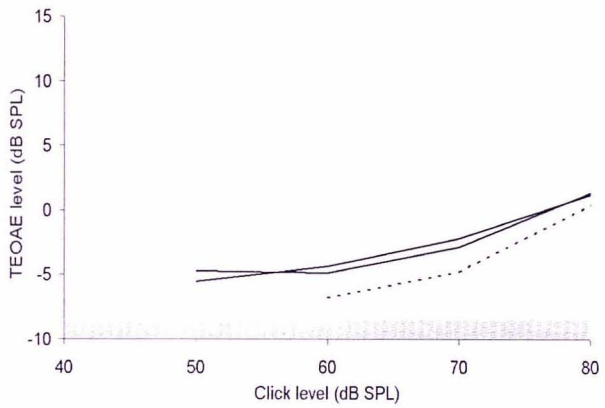
Figure 6-21: Mean TEOAE level over the seven test sessions. A) Broadband response. B) 1 kHz. C) 2 kHz. D) 3 kHz. E) 4 kHz. Key to symbols - click level crosses: 80 dB, triangles: 70 dB, diamonds: 60 dB, squares: 50 dB. Arrow shows the period of salicylate consumption. Mean noise floor shown by shaded area.



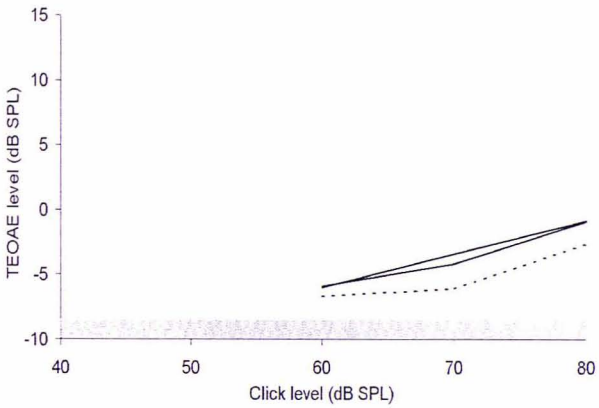
A) Broadband response



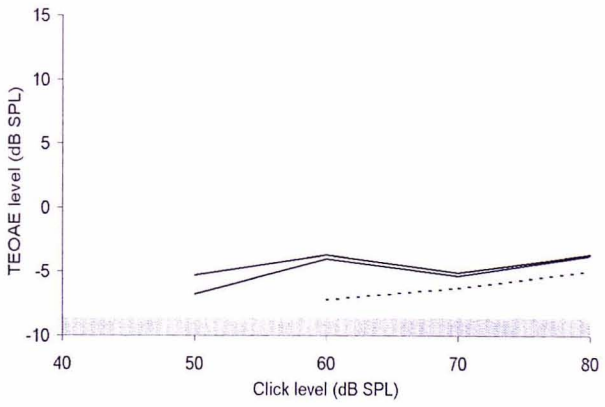
B) 1 kHz



C) 2 kHz



D) 3 kHz



E) 4 kHz

Figure 6-22: Mean TEOAE I/O functions (left and right ears combined). A) Broadband response. B) 1 kHz. C) 2 kHz. D) 3 kHz. E) 4 kHz. Key – solid lines: sessions 1 and 2, broken lines: session 5.

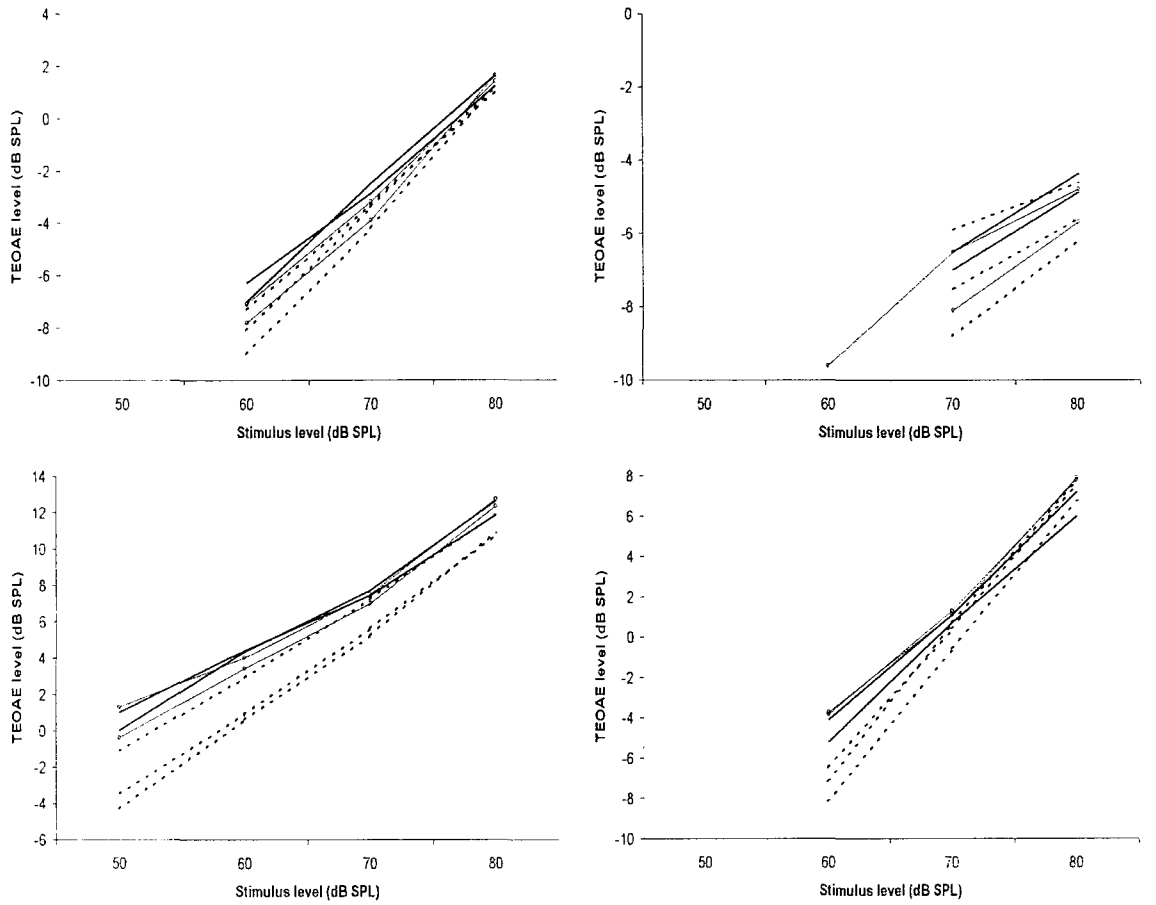


Figure 6-23: Example TEOAE I/O functions pre-, peri- and post-salicylate. A) Subject 16, left ear, 3 kHz. B) Subject 4, left ear, 4 kHz. C) Subject 6 right ear, broadband response. D) Subject 8, right ear 1 kHz. Key – solid lines: sessions 1 & 2, dashed lines: sessions 3, 4 & 5, sold lines with circle symbols: sessions 6 &7.

To assess the change in linearity of the I/O functions with salicylate consumption, slopes of the functions were calculated between stimulus levels of 60 to 80 dB using linear regression. The results comparing the median slopes of the I/O function from sessions 1 and 2 and session 5 are shown in Table 6-11.

Table 6-11: Median slope values of the TEOAE I/O functions (dB/dB)

Session	Frequency (kHz)			
	BB	1	2	3
Mean 1 & 2	0.52	0.54	0.44	0.37
5	0.61	0.67	0.56	0.45

BB: broadband

The results show a median increase in the slope of the I/O function with salicylate consumption of approximately 0.1-0.15 dB/dB. These changes are consistent with the framework, in which there is a differential reduction in TEOAE level at the lower intensity stimulus levels. However there were individual ears that showed an equal reduction in TEOAE at high stimulus levels.

6.6.6.2 TEOAE level and HTL

Group analysis

Using individual I/O functions, the change in TEOAE level with salicylate consumption at each stimulus level was estimated for each subject. It was predicted from the model that the changes in OAE level were related to HTL. Changes were calculated by comparing the mean I/O function from sessions 1 and 2 with that from session 5 for each subject. Results from session 5 were used as the TEOAE from this session were significantly different from the pre-salicylate sessions across the frequency range. Changes in TEOAE level were examined at stimulus levels of 60 and 70 dB. These stimulus levels were used as TEOAE at these levels were most sensitive to salicylate.

Changes in TEOAE level with aspirin consumption at these stimulus levels were in the range of + 1 dB to –10 dB. To assess whether the group changes in TEOAE level were significantly correlated with the changes in HTL, a correlation analysis was performed. The change in TEOAE level was assessed for correlation with the change in HTL at each frequency. Variables that were significantly correlated were then further analysed using linear regression (using TEOAE as the independent variable). Significant results are shown in Table 6-12.

This showed that although there were nine TEOAE measures that were significantly correlated with HTL, five of these were based on ten or less data points. This was mainly due to the problem of recording changes in TEOAE level at or below the noise floor. Also the restriction on the maximum salicylate dosage that could be used meant that most changes in HTL were 10 dB or less, and only a few subjects showed changes greater than this. This had implications for the correlation analysis as most data was clustered towards the normal hearing end of the range. The significant correlations

between the change in TEOAE and HTL were mostly at HTL frequencies of 1 or 2 kHz. The variables with correlation coefficient values of 0.7 and above were significant at $P<0.05$ level only.

There was an overall trend of a reduction in TEOAE level associated with an increase in HTL. However there was wide variability in the data and several points show changes in TEOAE, sometimes as great as 5 dB that were accompanied by little or no change in HTL. The correlations between changes in TEOAE level and HTL were low, although several showed a significant relationship with a change in HTL at 1 kHz.

Table 6-12: Results of the group correlation analysis relating the change in TEOAE level (independent variable) and the change in HTL (dependent variable)

TEOAE frequency (kHz)	HTL frequency (kHz)	Level (dB)	Correlation coefficient	Slope	R-square
BB	1	60	−0.44* (28)	−0.96	0.20
2	1	60	−0.76 * (10)	−1.77	0.58
	1	70	−0.41* (24)	−1.04	0.17
	2	60	−0.77** (10)	−1.36	0.60
	3	70	−0.42 (24)	−1.32	0.18
	4	60	−0.84* (10)	−2.08	0.70
3	1	60	−0.93* (5)	−2.68	0.87
	1	70	−0.50* (21)	−0.97	0.25
	2	60	−0.99* (5)	−1.57	0.98

*Key: $P\leq0.05$ *, $P\leq0.01$**, $P\leq0.005$***. The number of data points used in the analysis is shown in parentheses. BB: broadband.*

Individual subject analysis

The relationship between TEOAE level and HTL was then analysed within each individual subject over seven sessions. Correlation coefficient analysis was used to examine the relationship between HTL and TEOAE for each subject, ear, frequency and level.

As for the DPOAE results, most subjects showed highly significant correlations between TEOAE level and HTL in both left and right ears for varying combinations of variables. This showed that the changes in TEOAE closely paralleled the results of the HTL changes. Ears with significant relationships had correlation coefficient values of 0.7 and greater, indicating a close association

between the two variables. Most subjects had highly significant relationships between HTL and at least one TEOAE variable. Significant relationships were observed across the click level range, between 50 to 80 dB SPL. As was observed for the DPOAE results, there were differences between subjects and ears in terms of which TEOAE variable was correlated with which HTL variable. Figure 6-24 gives four examples of the close relationship between TEOAE and HTL.

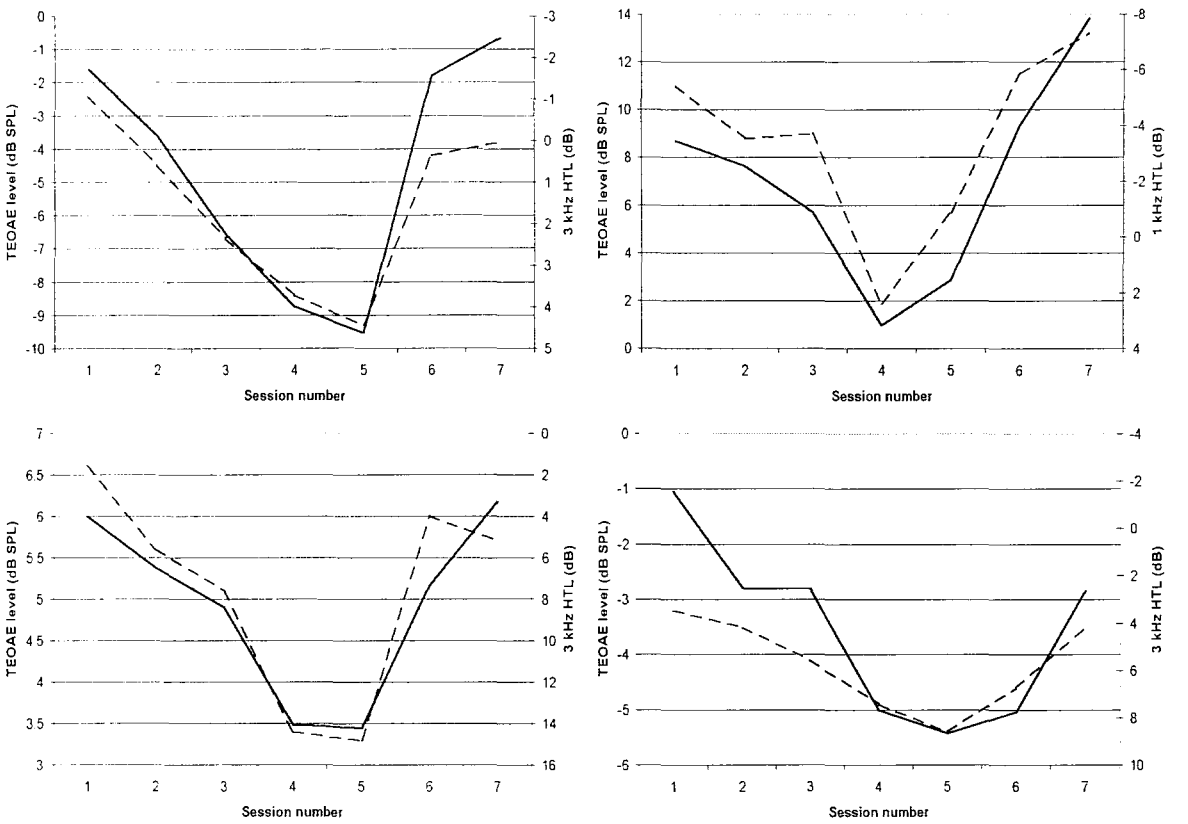


Figure 6-24: Association between TEOAE level and HTL with salicylate consumption. The axes were scaled so that TEOAE level and HTL were overlaid. A) Subject 8, left ear (TEOAE BB response, 50 dB). B) Subject 13, left ear (TEOAE BB response, 70 dB). C) Subject 9, left ear (TEOAE BB response, 70 dB). D) Subject 4, right ear (TEOAE 3 kHz, 70 dB). Key – solid line: HTL, dashed line: TEOAE level. All TEOAE measures were greater than the noise floor.

For each significant correlation, the time course of changes in TEOAE and HTL was examined visually to determine whether the change in TEOAE paralleled the change in HTL, or whether the changes in TEOAE preceded the changes in HTL. This showed that most changes were simultaneous and the changes in TEOAE preceded the changes in HTL in only 13% of all correlations.

The number of significant correlations between TEOAE and HTL frequencies within subjects and ears was calculated. Five different HTL frequencies were correlated with five different TEOAE frequencies, and the number of significant associations between TEOAE and HTL frequencies for each ear was calculated. For each TEOAE/HTL frequency combination, a significant association was

deemed present if there were significant correlations measured at more than one stimulus level for the TEOAE frequency in question.

Table 6-13 summarises the number of significant relationships between TEOAE and HTL for each subject and ear. As for DPOAE, this shows a range of the number of significant correlations between subjects and ears. Figure 6-25 compares the number of significant correlations across ears, which shows a weak relationship between the ears.

Table 6-13: Summary of the number of significant correlations (P<0.05) between HTL and TEOAE variables (out of a maximum of 25 correlations per ear)

Subject number	Left ear (max 25)	Right ear (max 25)	Left/right total (max 50)
1	0	0	0
2	0	0	0
3	1	2	3
4	0	6	6
6	4	0	4
7	9	8	17
8	4	2	6
9	12	3	15
10	1	5	6
11	0	0	0
12	4	3	7
13	9	4	13
14	1	2	3
15	0	0	0
16	0	4	4
17	0	8	8
19	1	0	1
25^{th} percentile	0	0	0
Median	1	2	1.5
75^{th} percentile	4	4	4

Figure 6-26 summarises the cumulative percentage of ears and subjects with significant correlations. There was a maximum of 25 possible TEOAE/HTL frequency combinations: therefore using a significance level of 0.05, 1/25 of these were likely to be due to chance. Approximately 50% of ears and 70% of subjects had two or more significant correlations between TEOAE and HTL.

The data were examined to determine which TEOAE and HTL frequencies were most likely to be significantly related.

Figure 6-27 summarises the number of significant correlations between the different variables for all ears. The highest number of significant relationship occurred between the broadband TEOAE level

and HTL at 3 kHz. HTL of 1, 2 3 and 4 kHz were most likely to be related to the broadband TEOAE. The higher HTL frequencies were most likely to be significantly related to TEOAE level at the higher frequencies.

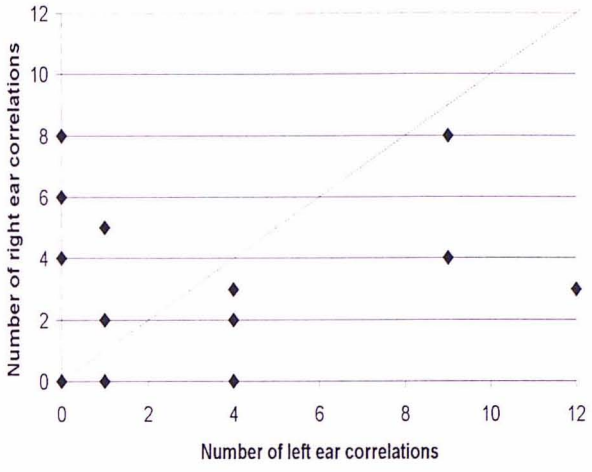


Figure 6-25: Relationship within subjects between the number of significant correlations (TEOAE/HTL) in the left and right ears. The dashed line shows a 1:1 relationship. There was a 27% correlation between the number of correlations in the left and right ears.

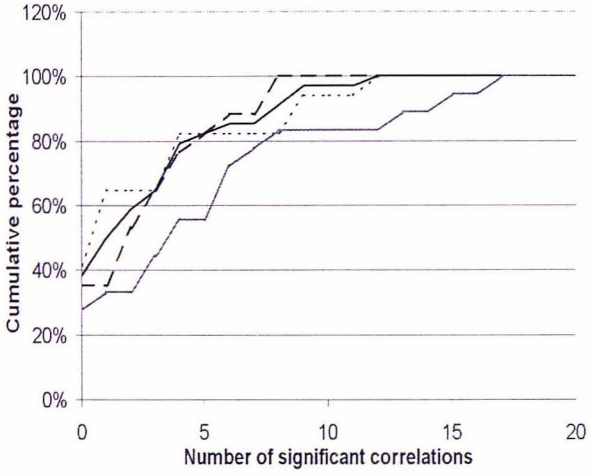


Figure 6-26: Cumulative % of ears/subjects with significant correlations. Key – dark solid line: left/right ears combined, dashed line: right ear, dotted line: left ear, grey solid line: subjects.

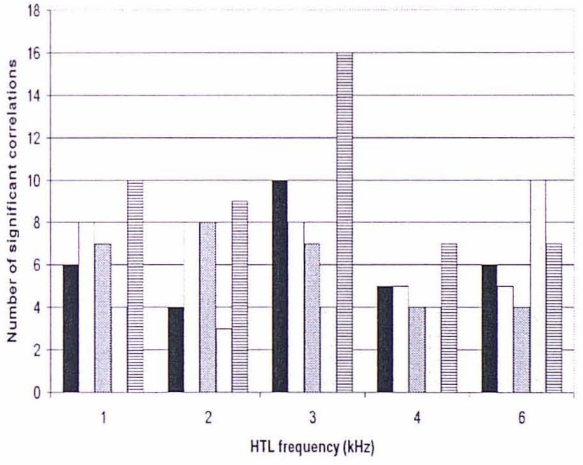


Figure 6-27: Summary of the frequency relationship of the significant correlations between HTL and TEOAE frequencies during salicylate consumption. Key: TEOAE frequency black bars: 1 kHz, white bars: 2 kHz, grey bars: 3 kHz, stippled bars: 4 kHz, striped bars: broadband response.

The data were examined in the same way as the DPOAE results to determine if there were factors that predicted those subjects and ears that showed highly significant relationships between TEOAE and HTL changes. Factors examined were similar to those examined for the DPOAE results: initial starting HTL, size of the change in TEOAE, and size of the change in HTL (at individual frequencies

and average frequencies). Analysis of these factors showed no marked effect of these on the relationship between TEOAE and HTL. Although there was no systematic relationship between the factors and the strength of the correlation, three subjects with high correlations and numerous relationships between TEOAE and HTL variables all had consistent changes in HTL of 5 dB or greater at several frequencies over sessions 3, 4 and 5 consecutively (subjects 7, 9 and 13).

In those ears with significant relationships between TEOAE and HTL, the individual relationships between the variables were examined in further detail. Figure 6-28 and Figure 6-29 illustrate the relationship between the change in HTL and the change in TEOAE for several subjects. There are similarities in the change in TEOAE with HTL for some subjects but there are also differences between subjects.

Using the results from the ears with significant relationships, median slope values were calculated for the relationship between the change in HTL and TEOAE at the different stimulus levels. These are summarised in Figure 6-30 at each frequency. Standard deviation bars are not shown for clarity, but were approximately 2.5 dB. This shows a consistent pattern of decreasing slope value with increasing stimulus level for the broadband responses and at 2 kHz. However the results at the other frequencies are more variable.

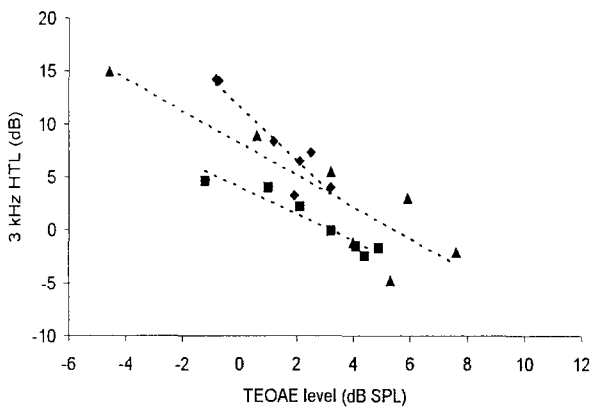


Figure 6-28: Broadband TEOAE level (60 dB) versus 3 kHz HTL across sessions. Key to symbols – squares: subject 8, diamonds: subject 9, triangles: subject 13.

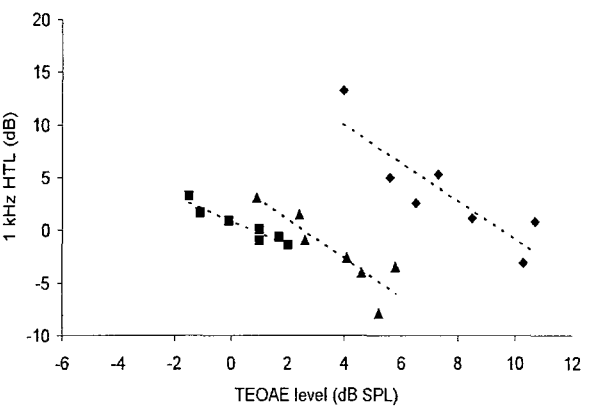


Figure 6-29: Broadband TEOAE level (60 dB) versus 1 kHz HTL across sessions. Key to symbols – squares: subject 4, diamonds: subject 7, triangles: subject 13.

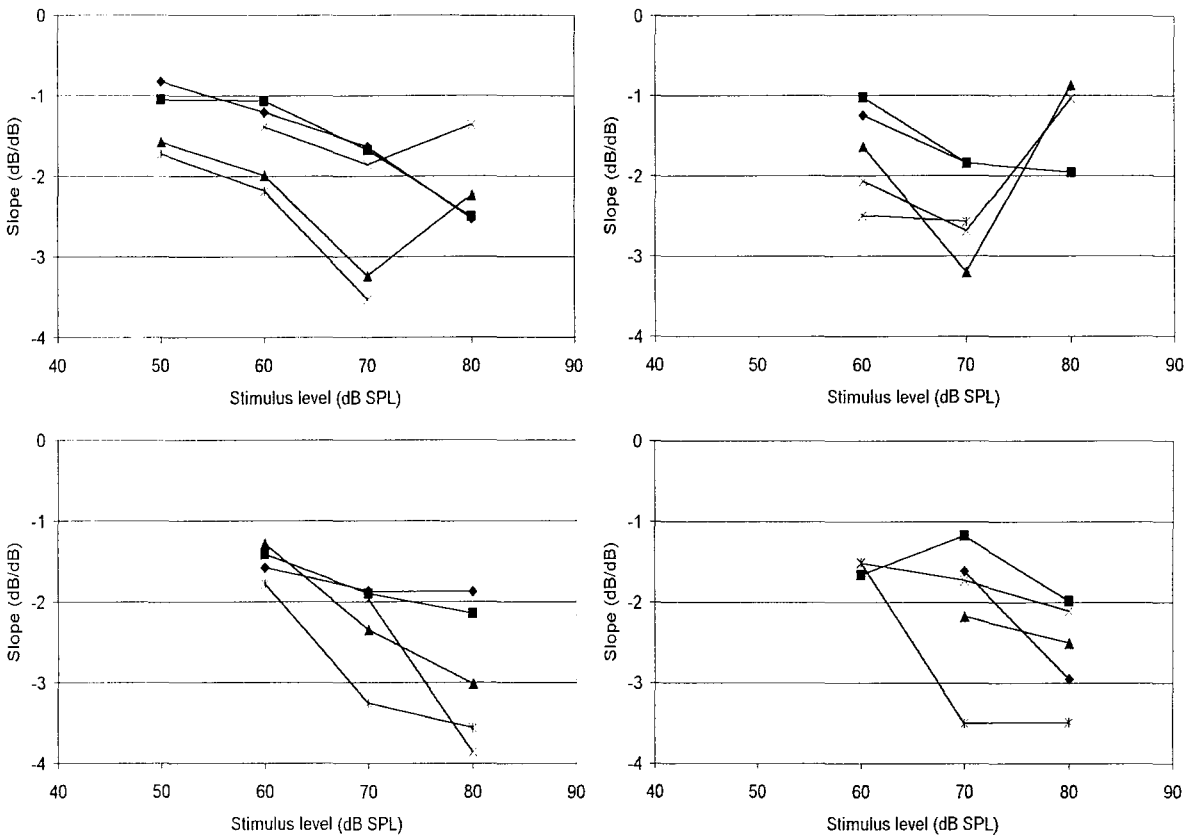


Figure 6-30: Median slope values of the relationship between TEOAE (independent variable) and HTL (dependent variable). Key to symbols – HTL frequencies - squares: 1 kHz, diamonds: 2 kHz, triangles: 3 kHz, crosses: 4 kHz, stars: 6 kHz.

6.6.6.3 TEOAE and SOAE

The TEOAE from ears with measurable SOAE were examined in more detail, in the same way as for DPOAE. The group was split into two: the SOAE positive (SOAE+) group contained all ears with measurable SOAE, and the SOAE negative (SOAE–) group contained all ears with no measurable SOAE.

Table 6-14 compares the mean pre-aspirin TEOAE levels from sessions 1 and 2 in the two groups. This showed a trend of larger TEOAE levels in the SOAE+ group at 1 and 2 kHz, and for the broadband responses. An independent samples t-test was used to test for significant differences at the lower intensity stimulus levels where TEOAE were recorded in most subjects. The differences were significant at 2 kHz, and for the broadband response. The differences reached borderline significance at 1 kHz, and were not significant at 3 and 4 kHz.

Table 6-14 also compares the changes in TEOAE between subjects in the SOAE+ and SOAE– groups and Figure 6-31 shows the TEOAE I/O functions pre- and peri-aspirin for the two groups. The two groups showed similar changes in TEOAE level with aspirin consumption, and using an independent

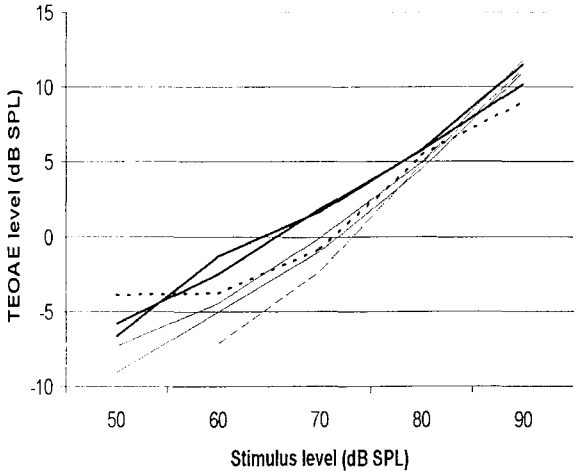
samples t-test, the differences between the groups at 70 and 80 dB stimulus levels were not significant.

Table 6-14: Comparison of the absolute TEOAE levels, and changes in TEOAE in the SOAE+ and SOAE– groups

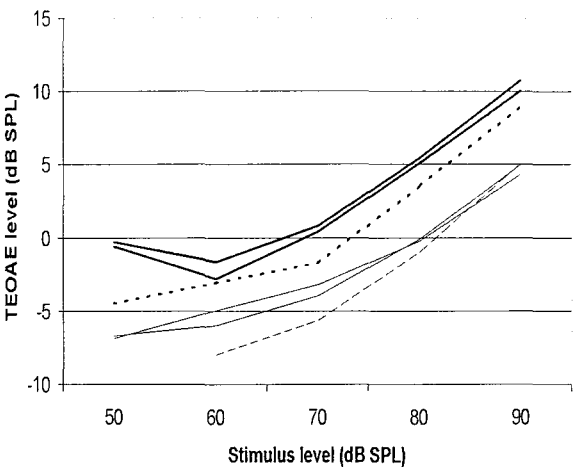
Frequency (kHz)	Stimulus level (dB SPL)	Mean TEOAE level sessions 1/2 (dB)		Significance value (P)	Mean change in TEOAE level from sessions 1/2 to session 5 (dB)		Significance value (P)
		SOAE+	SOAE –		SOAE+	SOAE –	
Broadband	60	6.100	2.112	0.01*	-3.514	-4.229	0.40
	70	9.928	7.050	0.05	-2.357	-2.758	0.58
1	60	-2.492	-4.705	0.09	-2.710	-2.984	0.72
	70	1.742	-0.462	0.14	-2.571	-2.136	0.62
2	70	0.621	-3.543	0.02*	-2.335	-2.805	0.47
	80	5.278	0.186	0.01*	-1.707	-1.293	0.59
3	70	-3.835	-3.350	0.78	-2.335	-3.384	0.22
	80	0.307	-0.781	0.31	-1.264	-2.168	0.17
4	70	-6.430	-4.780	0.48	-1.133	-2.420	0.59
	80	-4.107	-3.118	0.64	-0.578	-1.339	0.60

** $P<0.05$*

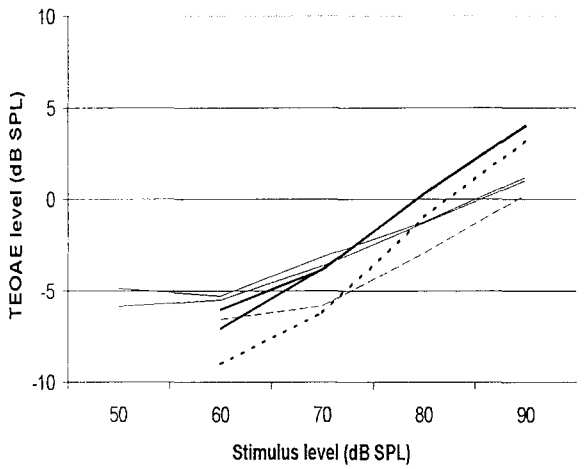
Also investigated was whether ears with SOAE more likely to have significant relationships between the change in TEOAE and HTL than ears with absent SOAE. As the data were not normally distributed, a Mann-Whitney U test was used to compare the number of significant correlations between the two SOAE groups. This showed no significant difference in the number of significant correlations between the SOAE+ and SOAE– groups (P=0.967).



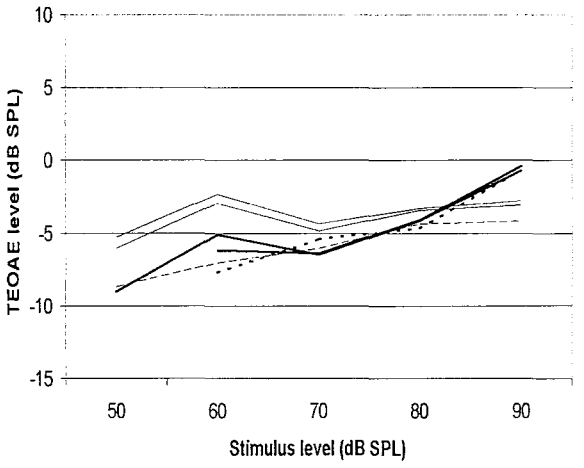
A) TEOAE 1 kHz



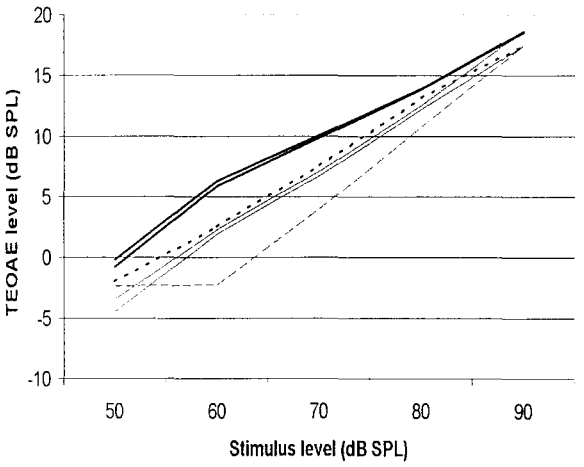
B) TEOAE 2 kHz



C) TEOAE 3 kHz



D) TEOAE 4 kHz



E) TEOAE 1 kHz

Figure 6-31: A comparison of TEOAE I/O functions from SOAE+ and SOAE– groups. A) 1 kHz. B) 2 kHz. C) 3 kHz. D) 4 kHz. E) Broadband. Key – thick dark lines: SOAE+ group, thin lines: SOAE– group, solid line: pre-aspirin sessions 1 and 2, dashed lines: peri-aspirin session 5.

6.6.7 MLS TEOAE

6.6.7.1 MLS TEOAE I/O functions

Results for the MLS TEOAE waveforms obtained at a click rate of 5000 clicks/s are described here. MLS TEOAE at each click level were filtered into 1/6-octave bands and MLS TEOAE level was calculated for each frequency band, and also for the broadband response.

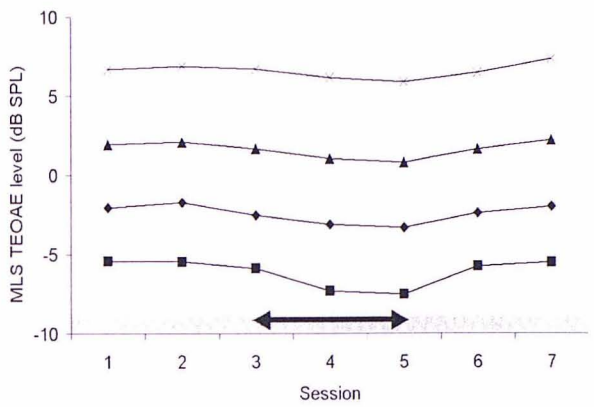
Changes in MLS TEOAE level were examined. Figure 6-32 shows the mean MLS TEOAE level shifts across sessions 1 to 7, plotted by click level. Data combine both left and right ears.

This shows a reduction in MLS TEOAE level with salicylate, dependent on stimulus level. MLS TEOAE evoked by 80 dB clicks were relatively insensitive to salicylate, and showed little or no change in level. MLS TEOAE evoked by lower click levels were more sensitive to salicylate, with MLS TEOAE evoked by clicks of 60 and 50 dB showing the greatest reduction in level. Unlike the TEOAE results, it was possible to record emission to a click level of 50 dB, and this level was the most sensitive and showed the biggest changes in level with salicylate. Salicylate had the smallest effect on the broadband responses and at 1 kHz. Responses at 2 and 3 kHz showed the largest changes in level.

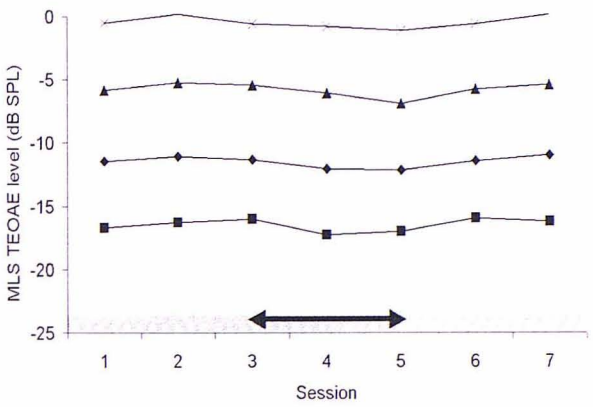
The levels of the 5000 clicks/s MLS TEOAE responses were approximately 10 dB lower than those obtained at the conventional rate of 50 clicks/s. The noise floor was also markedly lower at approximately –25 dB, at least 10 dB lower than the ILO288. This enabled responses at lower levels to be recorded and reduced noise floor problems.

A repeated measures ANOVA was performed to assess the effect of the within-subject factors: session, frequency and stimulus level on MLS TEOAE level. This showed a significant effect of session ($P<0.001$), frequency ($P<0.001$) and level ($P<0.001$).

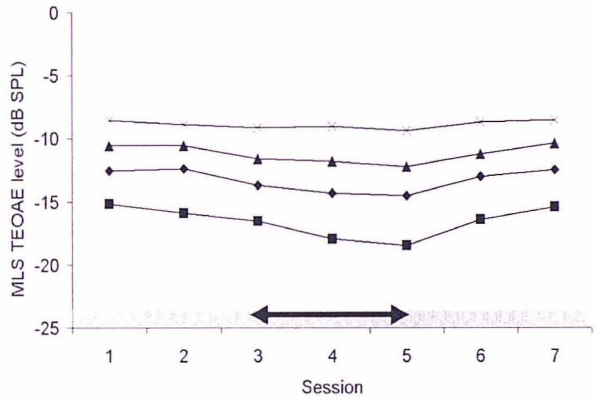
According to the framework, salicylate consumptions is expected to result in an increase in I/O function slope along with a reduction in level. I/O functions were plotted at each frequency. Mean changes in the I/O function (calculated across subjects and ears) from pre-aspirin to session 5 are shown in Figure 6-33.



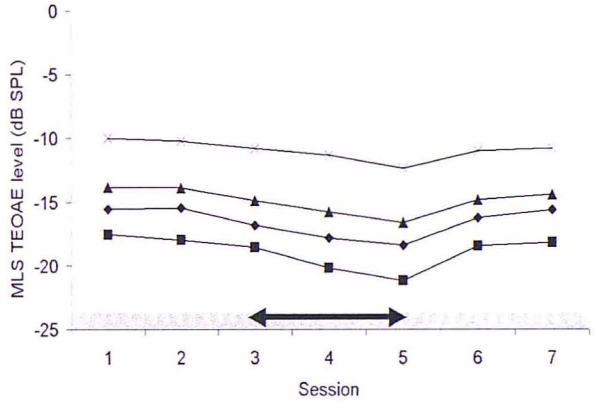
A) Broadband response



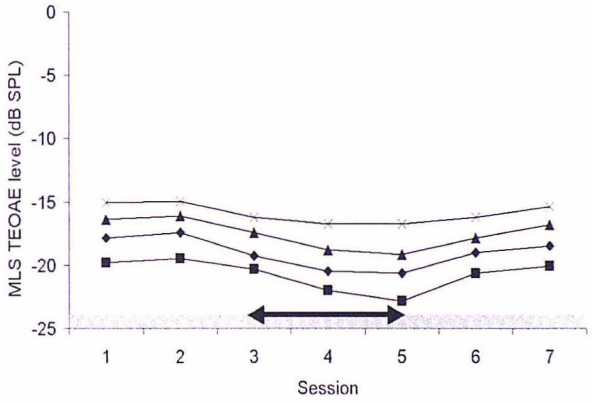
B) 1 kHz



C) 2 kHz

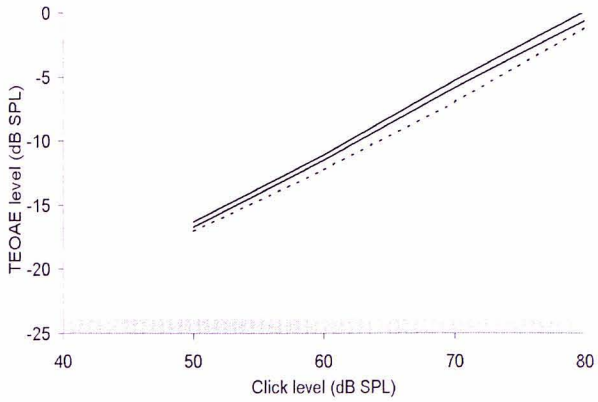


D) 3 kHz

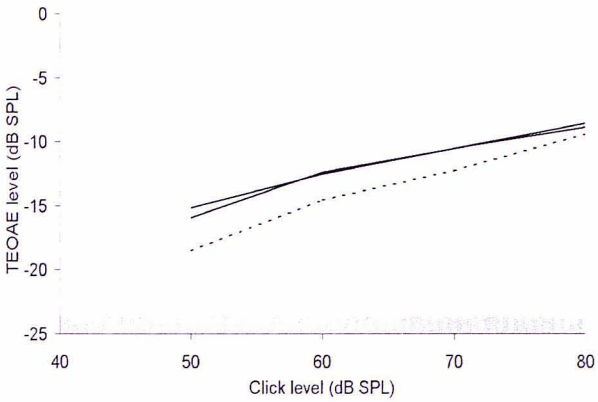


E) 4 kHz

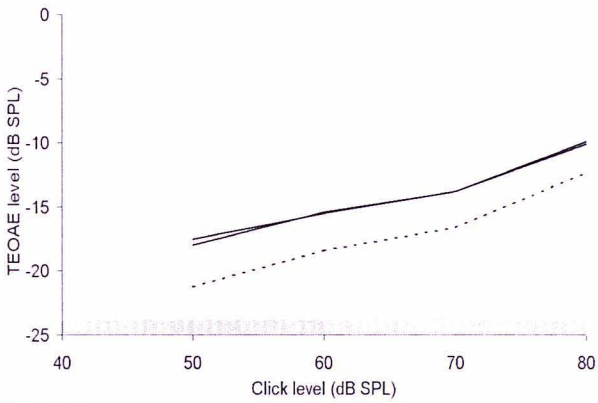
Figure 6-32: Mean MLS TEOAE level (5000 clicks/s) over the seven test sessions. A) 1 kHz. B) 2 kHz. C) 3 kHz. D) 4 kHz. E) Broadband response. Key to symbols – crosses: 80 dB, triangles: 70 dB, diamonds: 60 dB, squares: 50 dB. Arrow shows the period of salicylate consumption. Mean noise floor shown by shaded area.



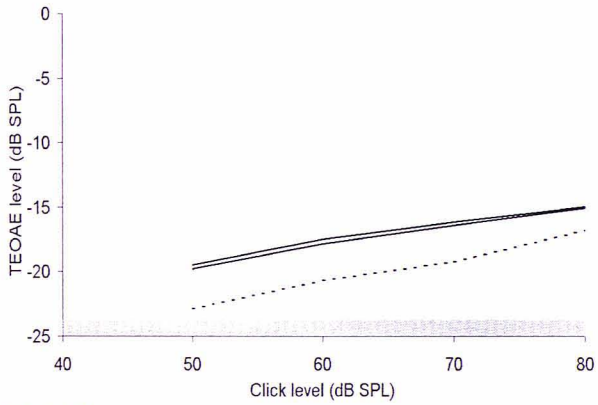
A) 1 kHz



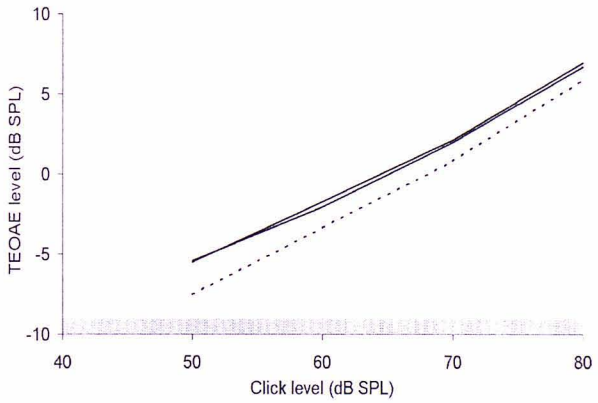
B) 2 kHz



C) 3kHz



D) 4 kHz



E) Broadband response

Figure 6-33: Mean MLS TEOAE I/O functions at 5000 clicks/s (left and right ears combined). A) 1 kHz. B) 2 kHz. C) 3 kHz. D) 4 kHz. E) Broadband response. Key – solid lines: sessions 1 and 2, broken lines: session 5. Mean noise floor shown by shaded area.

The I/O functions showed similar changes with salicylate consumption, comparable with the conventional TEOAE. Again, there were no substantial changes at 1 kHz. The function at 2 kHz showed a right shift, with the greatest changes at the lower intensity levels, indicating an overall reduction in cochlear nonlinearity. At 3 kHz, the function showed a downward shift with similar changes in level at all click levels. There was variation between subjects and ears in the responses of the I/O functions to aspirin. Figure 6-34 gives examples of the different responses to salicylate of different subjects.

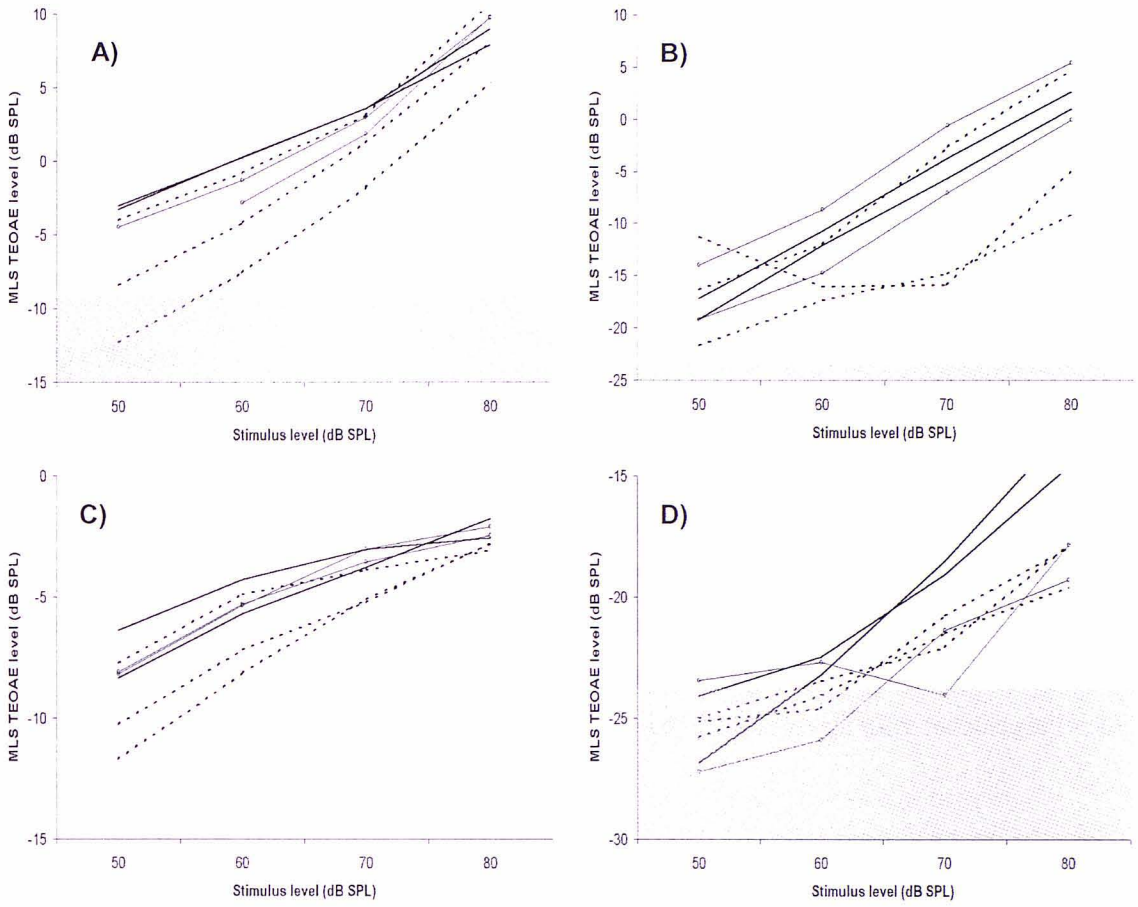


Figure 6-34: MLS TEOAE I/O functions. A) Subject 7, right ear, broadband response. B) Subject 13, left ear, 1 kHz. C) Subject 3 right ear, 2 kHz. D) Subject 2, right ear 3 kHz. Key – solid lines: sessions 1 & 2, dashed lines: sessions 3, 4 & 5, solid lines with circle symbols: sessions 6 &7. Shaded area shows the mean noise floor.

To assess the change in linearity of the I/O functions with salicylate consumption, slopes of the functions were calculated between stimulus levels of 60 and 80 dB using linear regression. The results of the median slopes of the I/O function from sessions 1 and 2 and session 5 are shown in Table 6-15.

Table 6-15: Median slope values of the MLS TEOAE I/O functions (dB/dB)

Session	Frequency (kHz)				
	BB	1	2	3	4
Mean 1 & 2	0.45	0.54	0.14	0.26	0.14
5	0.48	0.58	0.23	0.28	0.19

BB: broadband

The median slope results of the MLS TEOAE I/O functions showed a small increase in the I/O function slope for the broadband response and at 2 and 3 kHz of 0.03 to 0.07 dB/dB. There was no mean change at 1 kHz. These results are not fully consistent with the framework and show that there are changes in MLS TEOAE level across the stimulus range, rather than just at the lower intensity stimulus levels.

6.6.7.2 MLS TEOAE level and HTL

Group analysis

Using individual I/O functions, the change in MLS TEOAE level with salicylate consumption at each stimulus level was estimated for each subject. It was predicted from the model that the changes in OAE level were related to HTL. Changes were calculated by comparing the mean I/O function from sessions 1 and 2 with that from session 5 for each subject. Results from session 5 were used as the MLS TEOAE from this session were significantly different from the pre-salicylate sessions across the frequency range. Changes in MLS TEOAE level were examined at stimulus levels of 50, 60 and 70 dB. These stimulus levels were used, as MLS TEOAE at these levels were most sensitive to salicylate.

Changes in MLS TEOAE at these stimulus levels were in the range + 7dB to –12 dB. To assess whether the group changes in MLS TEOAE level were significantly correlated with the changes in HTL, a correlation analysis was performed. The change in MLS TEOAE level was assessed for correlation with the changes in HTL across the frequency range. Variables that were significantly associated were then further analysed using linear regression (using MLS TEOAE as the independent variable). Significant results are shown in Table 6-16.

As was observed for the conventional TEOAE, the significant correlations were found mainly between MLS TEOAE and the lower HTL frequencies. The significant correlations between MLS TEOAE level and HTL were mostly at HTL frequencies of 1 or 2 kHz. No relationship was observed at the higher HTL frequencies. MLS TEOAE level at 2 kHz (60 dB stimulus level) and HTL showed the highest correlations, with a correlation coefficient of 0.68 and R-square value of 0.46. Examples of the highest correlations are shown in Figure 6-35.

These showed an overall trend of a reduction in MLS TEOAE level associated with an increase in HTL. However there was wide variability in the data. Both figures include examples showing changes in MLS TEOAE level accompanied by little or no change in HTL.

Table 6-16: Results of the correlation analysis relating the change in MLS TEOAE level (independent variable) with the change in HTL (dependent variable)

TEOAE frequency (kHz)	HTL frequency (kHz)	Click level (dB)	Correlation coefficient (R)	Slope	R-square
BB	1	50	−0.41* (32)	−0.51	0.17
	1	60	−0.54** (32)	−0.90	0.29
	1	70	−0.43* (32)	−0.90	0.18
	2	60	−0.39* (32)	−0.63	0.15
	6	60	−0.40* (32)	−1.19	0.16
2	1	50	−0.50** (32)	−0.49	0.25
	1	60	−0.68*** (32)	−1.02	0.46
	1	70	−0.47* (32)	−0.78	0.22
	2	50	−0.38* (32)	−0.36	0.14
3	1	50	−0.43* (32)	−0.49	0.19
	1	60	−0.42* (32)	−0.52	0.18
	1	70	−0.39* (32)	−0.57	0.15
	2	50	−0.37* (32)	−0.42	0.14
	2	60	−0.49*** (32)	−0.60	0.25

*The number of data points in the analysis is shown in parentheses. Key - *P≤0.05, **P≤0.01, ***P≤0.005. BB: broadband.*

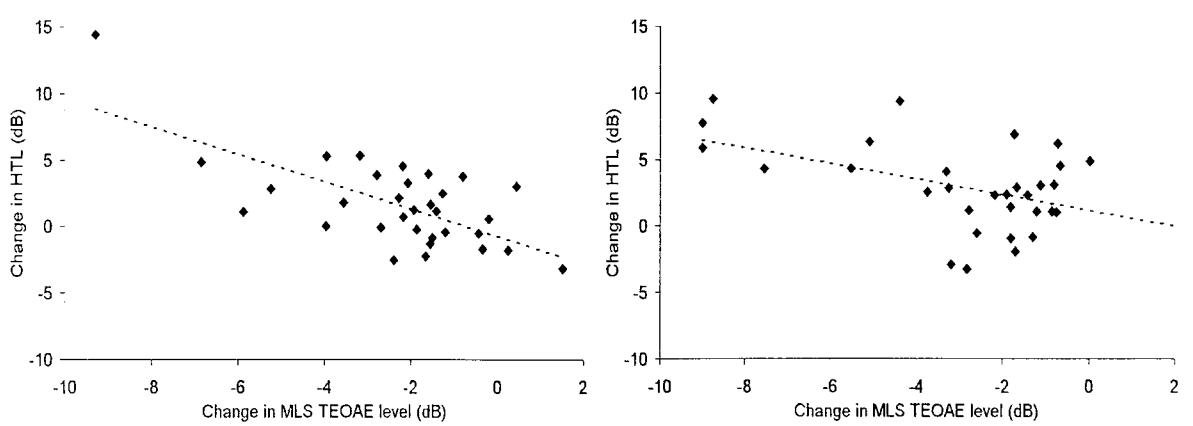


Figure 6-35: A) Change in MLS TEOAE level at 2 kHz (60 dB stimulus level) plotted against the change in HTL at 1 kHz. B) Change in MLS TEOAE level at 3 kHz (60 dB stimulus level) plotted against the change in HTL at 2 kHz. Linear regression line plotted.

Individual subject analysis

The relationship between MLS TEOAE level and HTL was then analysed within each individual subject over seven sessions. Correlation analysis was used to examine the relationship between HTL and MLS TEOAE for each subject, ear, frequency and level.

Most subjects and ears showed highly significant correlations between the MLS TEOAE level and HTL over the seven-day period. This showed that the changes in MLS TEOAE closely paralleled the results of the HTL changes. Ears with significant relationships had correlation coefficient values of 0.7 and greater, indicating a close association between the two variables. Most ears had significant relationships between HTL and at least one MLS TEOAE variable. Significant relationships were observed across the click level range, between 50 to 80 dB SPL. There were differences between ears in terms of which MLS TEOAE variable was correlated with which HTL variable. Figure 6-36 gives four examples of the highest correlations between MLS TEOAE and HTL.

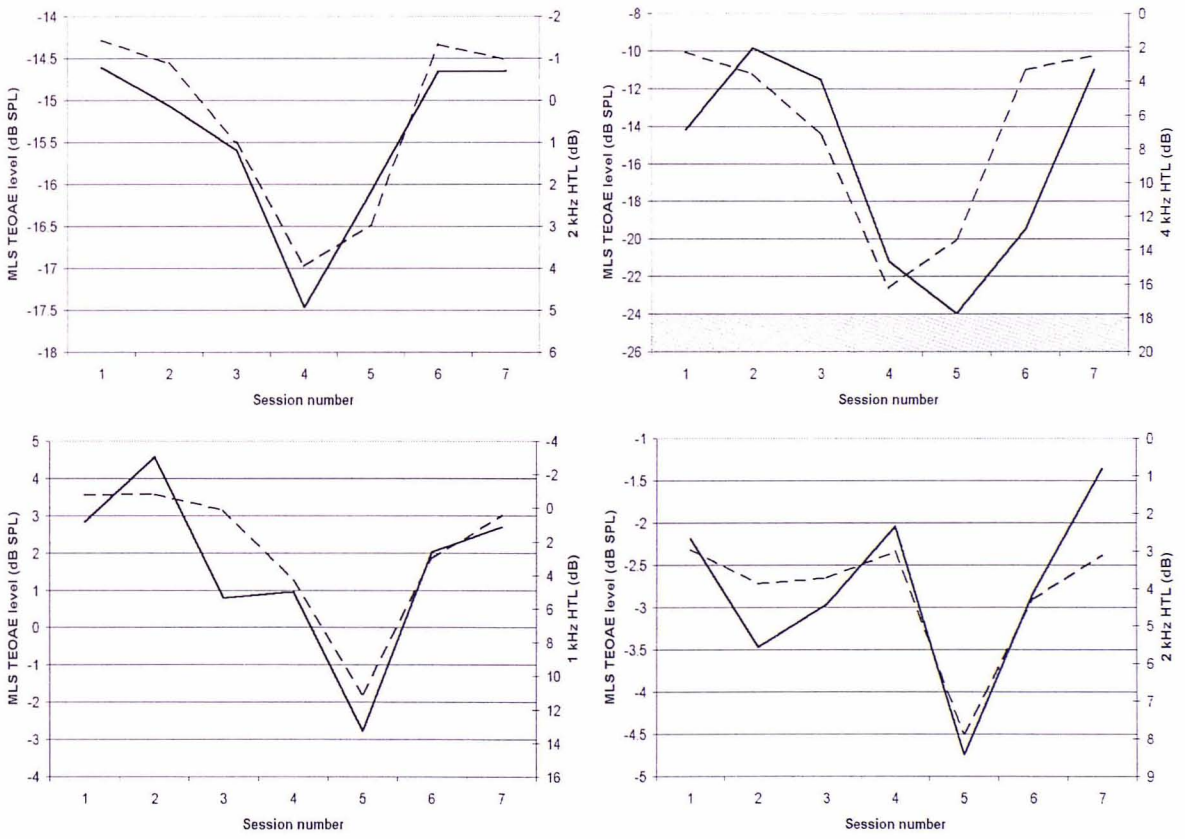


Figure 6-36: Association between MLS TEOAE level and HTL with salicylate consumption. A) Subject 9, left ear (MLS TEOAE 3 kHz response, 70 dB). B) Subject 13, left ear (MLS TEOAE, 3 kHz, 50 dB). C) Subject 7, right ear (MLS TEOAE broadband response, 70 dB). D) Subject 4, left ear (MLS TEOAE broadband response, 60 dB). Key – solid line: HTL, dashed line: MLS TEOAE level.

Each of the individual ear correlations was examined to determine whether the change in MLS TEOAE level preceded the change in HTL. This showed that most changes were simultaneous and the changes in MLS TEOAE preceded the changes in HTL in only 10% of all correlations.

The number of significant correlations between MLS TEOAE and HTL frequencies within subjects and ears was calculated. Five different HTL frequencies were correlated with five different MLS TEOAE frequencies, and the number of significant associations between MLS TEOAE and HTL frequencies for each ear was calculated. For each MLS TEOAE/HTL frequency combination, a significant association was deemed present if there were significant correlations measured at more than one stimulus level for the MLS TEOAE frequency in question.

Table 6-17 summarises the number of significant relationships between MLS TEOAE and HTL for each subject and ear. As for DPOAE, this showed a range of the number of significant correlations across subjects and ears. Figure 6-37 compares the number of significant correlations across ears, which shows more significant correlations in the left ear.

Table 6-17: Summary of the number of significant correlations (P<0.05) between HTL and MLS TEOAE (up to a maximum of 25 per ear)

Subject number	Left ear (max 25)	Right ear (max 25)	Left/right total (max 50)
1	1	0	1
2	2	2	4
3	1	1	2
4	4	3	7
6	0	0	0
7	9	13	22
8	2	1	3
9	5	1	6
10	4	8	12
11	0	0	0
12	2	2	4
13	12	5	17
14	6	1	7
15	3	0	3
16	3	0	3
17	0	0	0
19	3	1	4
25%	1	0	0.25
Median	3	1	3.5
75%	4	2	3.75

Figure 6-38 summarises the cumulative percentage of subjects according to the number of significant correlations. There was a maximum of 25 possible MLS TEOAE/HTL frequency combinations: therefore using a significance level of 0.05, 1/25 of these were likely to be due to chance. Approximately 50% of ears and 70% of subjects had two ore more significant correlation between MLS TEOAE and HTL.

The data were examined to determine which MLS TEOAE and HTL frequencies were more likely to be significantly related.

Figure 6-39 summarises the frequency of significant correlations between the different variables for all ears. The highest number of significant relationships was between MLS TEOAE level at frequencies of 2 and 3 kHz and HTL at 2 and 3 kHz. There were more significant relationships with MLS TEOAE evoked by lower stimulus levels than at the higher levels.

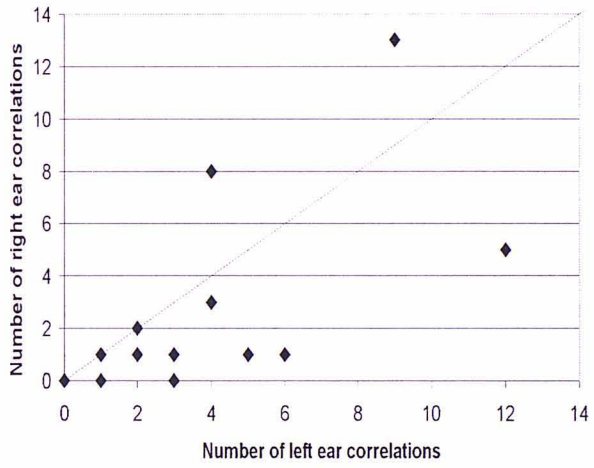


Figure 6-37: Relationship within subjects between the number of significant correlations (MLS TEOAE/HTL) in the left and right ears. The dashed line shows a 1:1 relationship.

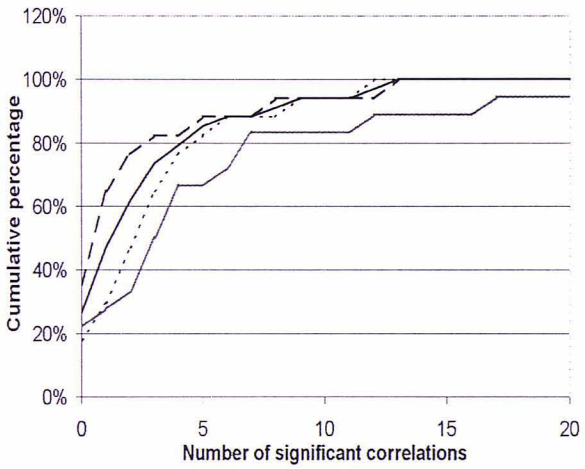


Figure 6-38: Cumulative % of ears/subjects with significant correlations. Key – dark solid line: left/right ears combined, dashed line: right ear, dotted line: left ear, grey solid line: subjects.

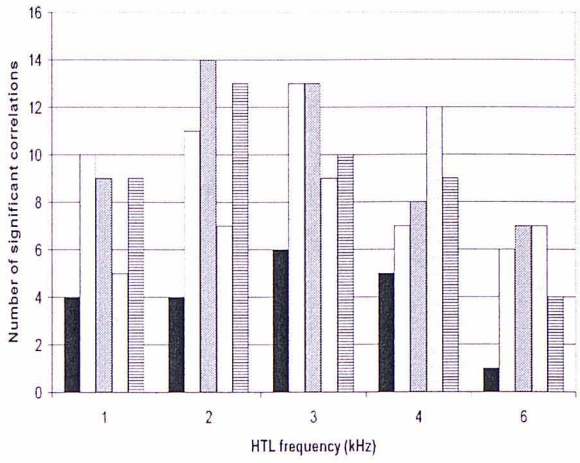


Figure 6-39: Summary of the frequency relationship of the significant correlations between HTL and MLS TEOAE frequencies during salicylate consumption. Key – MLS TEOAE frequency, black bars: 1 kHz, white bars: 2 kHz, grey bars: 3 kHz, dotted bars: 4 kHz, striped bars: broadband response.

The data were examined to determine if there were factors that predicted those ears that showed highly significant relationships between MLS TEOAE and HTL changes. Factors examined were: initial starting HTL, size of the change in MLS TEOAE, and size of the change in HTL (at individual frequencies and average frequencies). Analysis of these factors showed no marked effect of these on the relationship between MLS TEOAE and HTL.

In those ears with significant relationships between MLS TEOAE and HTL, the individual relationships between the variables were examined in further detail. Figure 6-40 and Figure 6-41 illustrate the relationship between the HTL and MLS TEOAE for several ears across sessions. These show that some ears show similarities in the relationship between the change in MLS TEOAE and the change in HTL. However each figure also shows at least one ear that has a markedly different relationship to the other ears.

The slope relating HTL and MLS TEOAE was calculated for each ear using linear regression. MLS TEOAE was used as the independent variable. Figure 6-42 summarises the median slope values relating the MLS TEOAE level and HTL across the sessions. Standard deviation bars are omitted.

This shows large variation in slope value particularly at the lower frequencies and for the broadband responses. The results at 3 kHz are less variable.

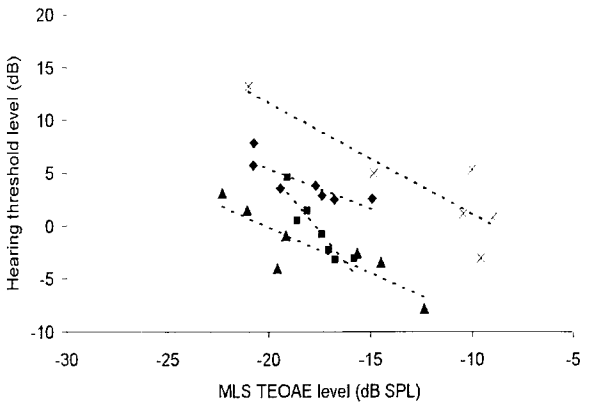


Figure 6-40: HTL at 1 kHz versus MLS TEOAE level at 2 kHz (50 dB) across sessions. Key to symbols – diamonds: subject 4, squares: subject 9, triangles: subject 13, crosses: subject 7.

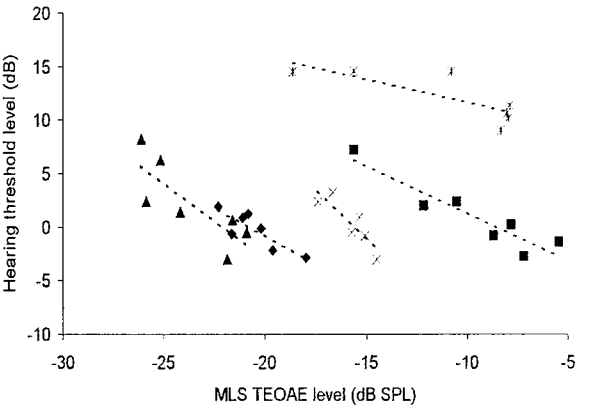


Figure 6-41: HTL at 2 kHz versus MLS TEOAE level at 3 kHz (60 dB) across sessions. Key to symbols – diamonds: subject 1, squares: subject 7, triangles: subject 10, crosses: subject 12, stars: subject 13.

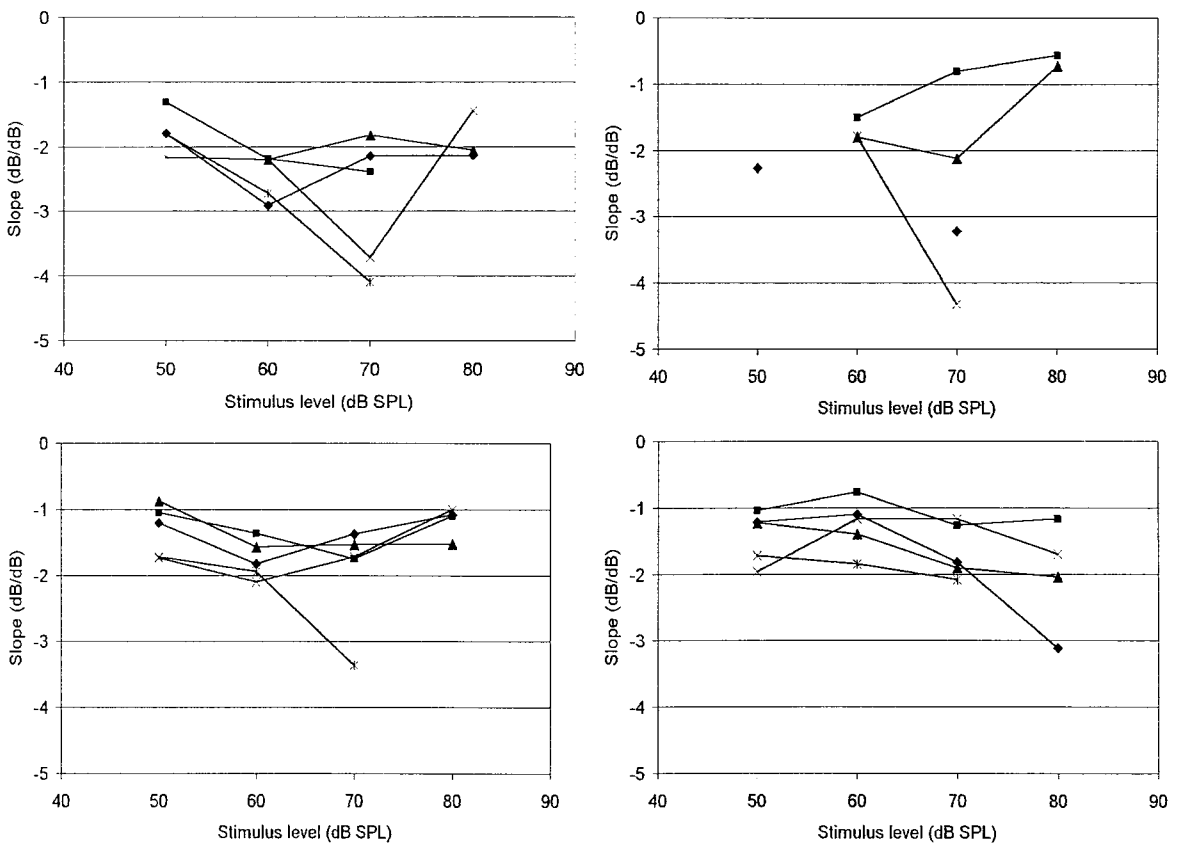


Figure 6-42: Median slope values of the relationship between MLS TEOAE (independent variable) and HTL (dependent variable). Key to symbols – HTL frequencies squares: 1 kHz, diamonds: 2 kHz, triangles: 3 kHz, crosses: 4 kHz, stars: 6 kHz.

6.6.7.3 MLS TEOAE and SOAE

The MLS TEOAE from those ears with measurable SOAE were examined in more detail, as for DPOAE and TEOAE. The group was split into two: the SOAE positive group (SOAE+) contained all ears with measurable SOAE, and the SOAE negative group (SOAE–) contained all ears with no measurable SOAE.

Table 6-18 compares the mean pre-aspirin MLS TEOAE levels from sessions 1 and in the SOAE+ and SOAE– groups. An independent samples t-test was used to test for significant differences. The broadband responses, and the MLS TEOAE at 2 kHz from the SOAE+ group had significant larger MLS TEOAE than those from the SOAE– group. Mean responses were also larger in the SOAE+ group at 1 kHz, but were only borderline significantly different to the SOAE– group. There was no statistically significant difference at 3 kHz.

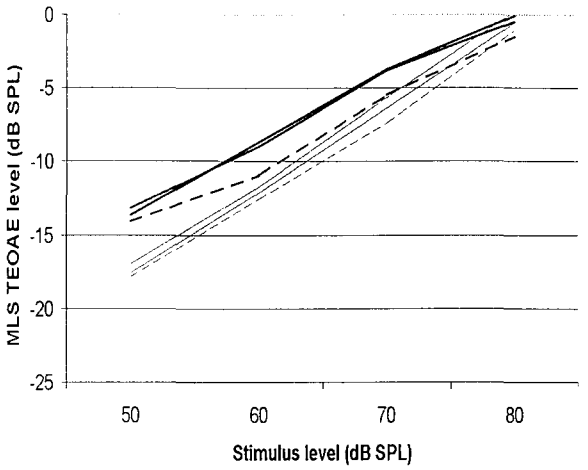
Table 6-18 also compares the changes in MLS TEOAE between the two groups and Figure 6-43 shows the MLS TEOAE I/O functions pre- and peri-aspirin for the two groups. An independent samples t-test was used to investigate whether the changes in DPOAE were significantly different between the SOAE+ and SOAE– groups. This showed no statistically significant difference, except at 3 kHz (60 dB stimulus level), where the SOAE– group showed a larger change with aspirin than the SOAE+ group.

Table 6-18: Comparison of the absolute MLS TEOAE levels, and changes in MLS TEOAE in the SOAE+ and SOAE– groups

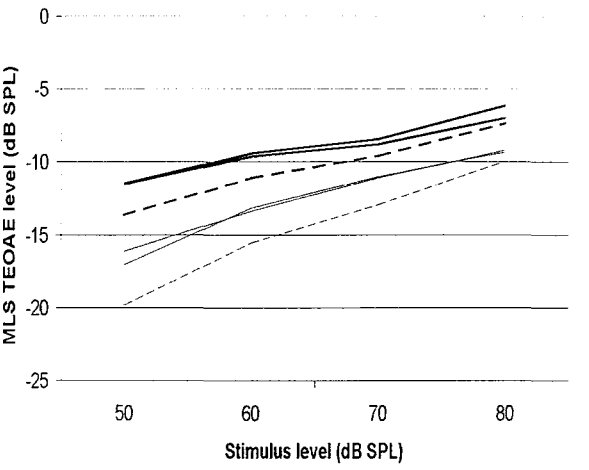
Frequency (kHz)	Stimulus level (dB SPL)	Mean TEOAE level sessions 1/2 (dB)		Significance value (P)	Mean change in TEOAE level from sessions 1/2 to session 5 (dB)		Significance value (P)
		SOAE+	SOAE –		SOAE+	SOAE –	
Broadband	50	-1.906	-6.284	0.00*	-1.490	-2.393	0.44
	60	1.134	-2.675	0.00*	-1.312	-1.627	0.71
1	50	-13.409	-17.128	0.07	-0.701	-1.081	0.74
	60	-8.828	-11.905	0.09	-2.073	-0.927	0.32
2	50	-11.500	-16.390	0.03*	-2.134	-3.669	0.29
	60	-9.522	-13.264	0.07	-1.545	-2.503	0.31
3	50	-17.392	-17.831	0.70	-2.430	-3.538	0.38
	60	-15.182	-15.549	0.74	-1.606	-3.133	0.03*

* $P<0.05$

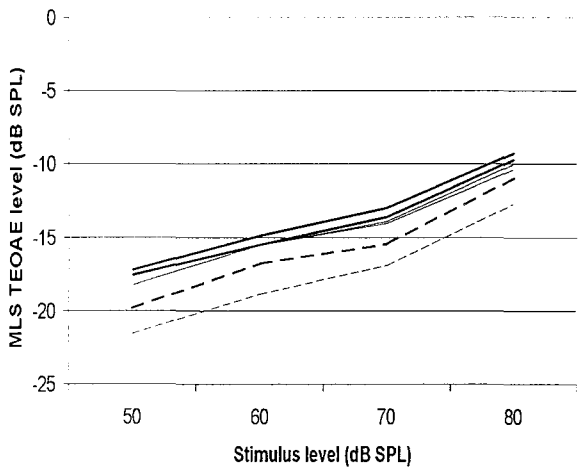
Also investigated was whether ears with SOAE more likely to have significant relationships between the change in MLS TEOAE and HTL than ears with absent SOAE. As the data were not normally distributed, a Mann-Whitney U test was used to compare the number of significant correlations between the two SOAE groups. This showed no significant difference in the number of significant correlations between the SOAE+ and SOAE– groups ($P=0.275$).



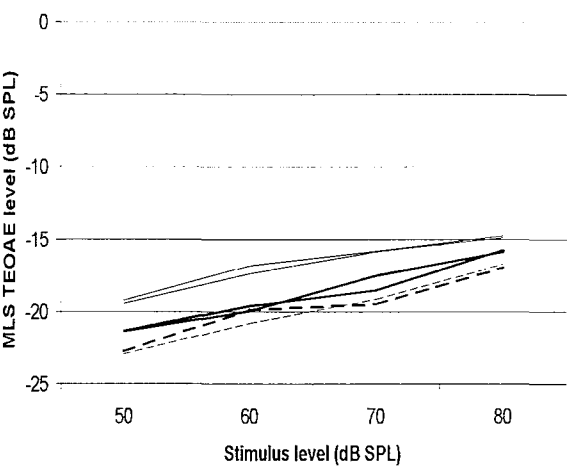
A) MLS TEOAE 1 kHz



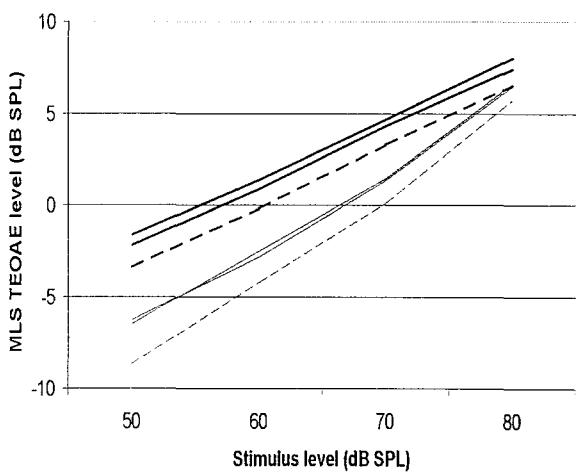
B) MLS TEOAE 2 kHz



C) MLS TEOAE 3 kHz



D) MLS TEOAE 4 kHz



E) MLS TEOAE Broadband

Figure 6-43: A comparison of MLS TEOAE I/O functions from SOAE+ and SOAE– groups. A) 1 kHz. B) 2 kHz. C) 3 kHz. D) 4 kHz. E) Broadband. Key – thick dark lines: SOAE+ group, thin lines: SOAE– group, solid line: pre-aspirin sessions 1 and 2, dashed lines: peri-aspirin session 5.

6.6.8 MLS TEOAE rate suppression

MLS TEOAE suppression was calculated for each frequency and click level, according to the framework described in Chapter 3. Changes in MLS TEOAE rate suppression with salicylate were examined. Figure 6-44 shows the mean MLS TEOAE rate suppression across sessions 1 to 7 for each frequency and click level. Data combine both the left and right ears.

Changes in rate suppression with salicylate were in the range + 8 to –10 dB. There was a general increase in suppression with increasing frequency. There was also an increase in suppression with decreasing level. With salicylate, there was a general reduction in rate suppression, dependent on frequency and stimulus level. This is likely to be related to the more compressive nature of the higher frequency I/O functions (see results of Experiment 1). The largest reduction in rate suppression occurred at 1 kHz frequency and the broadband response. At 1 kHz, the largest change was at a stimulus level of 80 dB. For the broadband response, all stimulus levels showed similar changes in rate suppression. Results at 2 and 3 kHz were more variable, although there was a slight reduction in rate suppression across the stimulus levels.

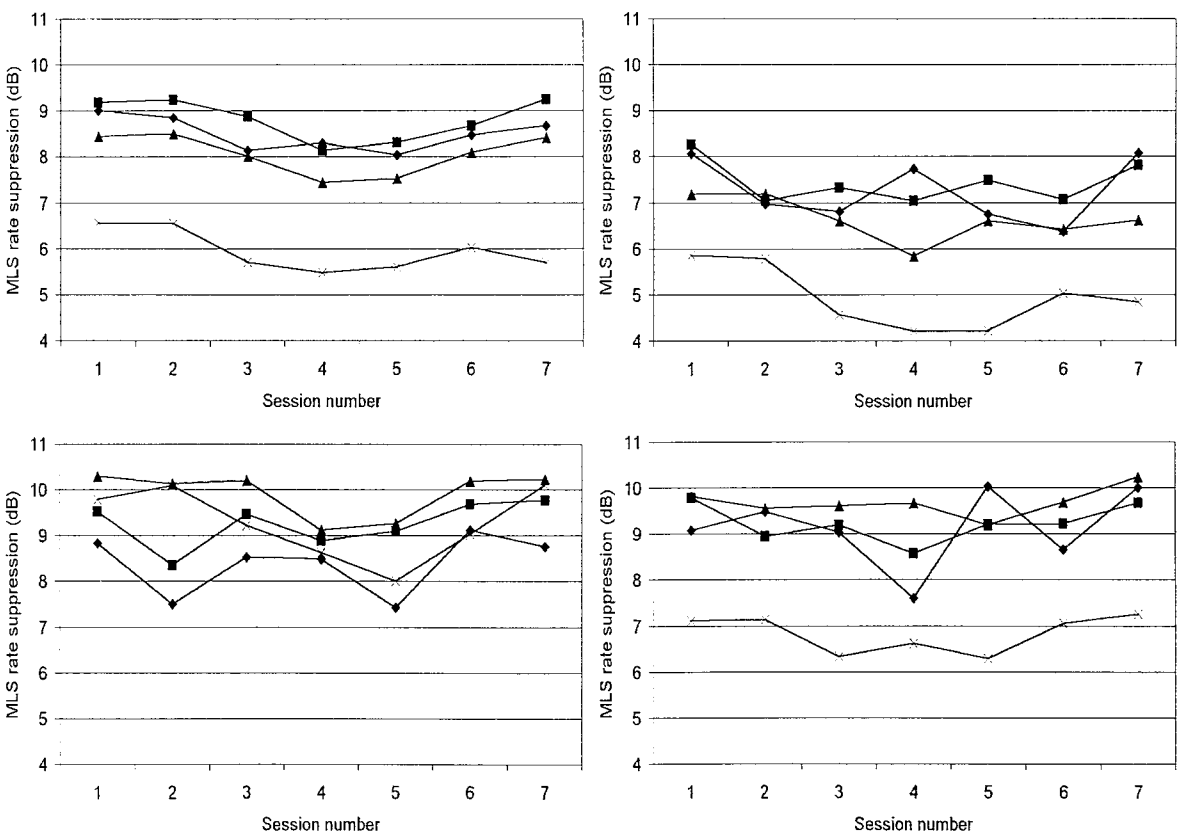


Figure 6-44: Mean MLS TEOAE rate suppression (S_{5000}) plotted across the sessions, at each click level. A) Broadband responses. B) 1 kHz. C) 2 kHz. D) 3 kHz. Key to symbols – diamonds: 50 dB, squares: 60 dB, triangles: 70 dB, crosses: 80 dB.

According to the model of Kapadia and Lutman (2001), salicylate consumption was expected to result in a reduction in rate suppression. Figure 6-45 shows the mean change in the rate-level functions (calculated across subjects and ears) from session 1 and 2 to session 5. Rate suppression can be calculated from these graphs as the difference in level between the two click rates.

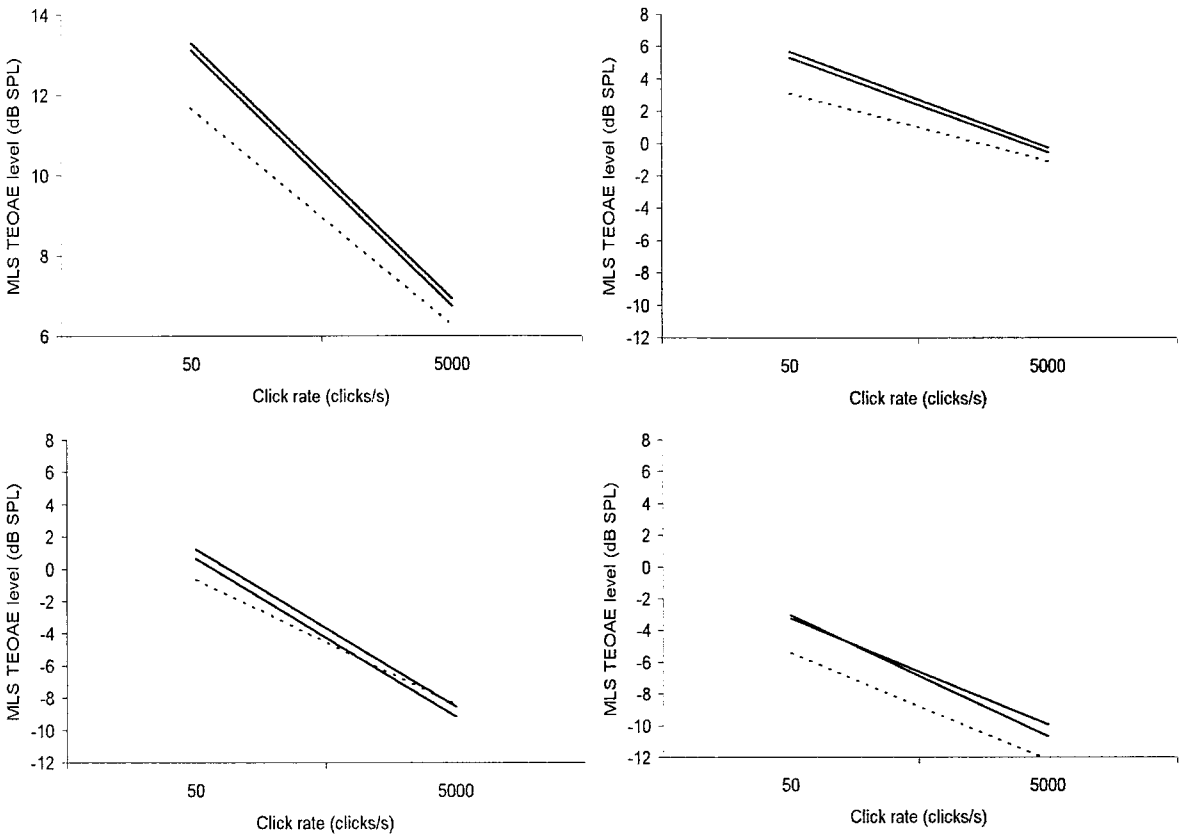


Figure 6-45: Mean MLS TEOAE rate/level functions (left and right ears combined). A) 1 kHz. B) 2 kHz. C) 3 kHz. D) Broadband response. Key – solid lines: sessions 1&2, broken line: session 5.

The rate-level functions showed different changes with salicylate consumption. At 1 and 2 kHz, and also for the BB response there was a change in the mean rate of MLS TEOAE level increase with decreasing click rate, i.e. a reduction in MLS TEOAE rate suppression. The largest change was at 1 kHz. This change in rate suppression was a result of a bigger change in level at 50 clicks/s. There was no mean change in rate suppression at 3 kHz.

Section 6.6.7.1described the median changes in the slopes of MLS TEOAE I/O functions with salicylate consumption. They showed little or no change in I/O function slope at 1 kHz, a small change for the broadband results, and the greatest change at 2 and 3 kHz. This indicates a reduction in compression with salicylate consumption at the higher frequencies.

6.6.8.1 MLS rate suppression and I/O function slope

Using the rate-level functions, the change in MLS rate suppression with salicylate consumption was estimated for each subject. It was predicted from the model that the change in rate suppression was associated with the change in the I/O function slope. An approximate 1 dB change in suppression with a 0.1 dB/dB change in slope was expected, as shown in Experiment 1.

Changes were calculated by comparing the mean rate-level function from sessions 1 and 2 with that from session 5 for each subject. Changes in rate suppression were analysed at a stimulus level of 80 dB, as this level was the most sensitive to differences in Experiment 1. The I/O function slope was calculated between stimulus levels of 60 and 80 dB, and the change was calculated as the difference between the mean of the pre-aspirin sessions (1 and 2) and session 5.

Visual analysis of the relationship between these variables showed that two outliers at 50 clicks/s had artificially improved the results of the correlation analysis. These outliers (both of which showed a change in I/O function slope of –0.3 dB/dB or less) were excluded and the analysis repeated. The restricted results of the group relationship between the change in I/O function slope and rate suppression are shown in Table 6-19. Figure 6-46 gives examples of the best relationships.

There were significant relationships between the change in I/O function slope and rate suppression for a small number of variables only. An increase in slope value was associated with a reduction in rate suppression, as predicted from the model. The significant relationships generally occurred between I/O function slope and rate suppression at the same frequencies i.e. the change in I/O function slope at 1 kHz was related to the change in rate suppression at 1 kHz. In most cases, the relationship between the two was as predicted from Experiment 1, i.e. 0.1 dB/dB change in slope was related to a 1 dB change in MLS suppression. It is likely that more significant relationships were not observed due to the small change in I/O function slope with salicylate.

Table 6-19: Correlation coefficients showing the relationship between the change in MLS rate suppression and the change in MLS TEOAE I/O function slope (sessions 1/2 and 5)

Rate suppression		I/O function slope							
		50 clicks/s				5000 clicks/s			
Freq (kHz)	Level (dB)	1	2	3	BB	1	2	3	BB
1	60	−0.55* (−8.9)		−0.53* (−19.1)					
	70								−0.44* (−10.3)
	80								
2	60		−0.62*** (−8.1)						
	70		−0.62*** (−8.9)				−0.42* (−5.2)		
	80						−0.53*** (−9.7)		
3	60								
	70								
	80						−0.42* (−5.6)	−0.57*** (−10.8)	
BB	60		−0.53* (−10.5)	−0.46* (−14.9)					
	70								−0.52*** (−5.7)
	80								−0.63*** (−7.8)

*Slope values shown in parentheses. Key: P≤0.05,** P≤0.01,*** P≤0.005. BB broadband*

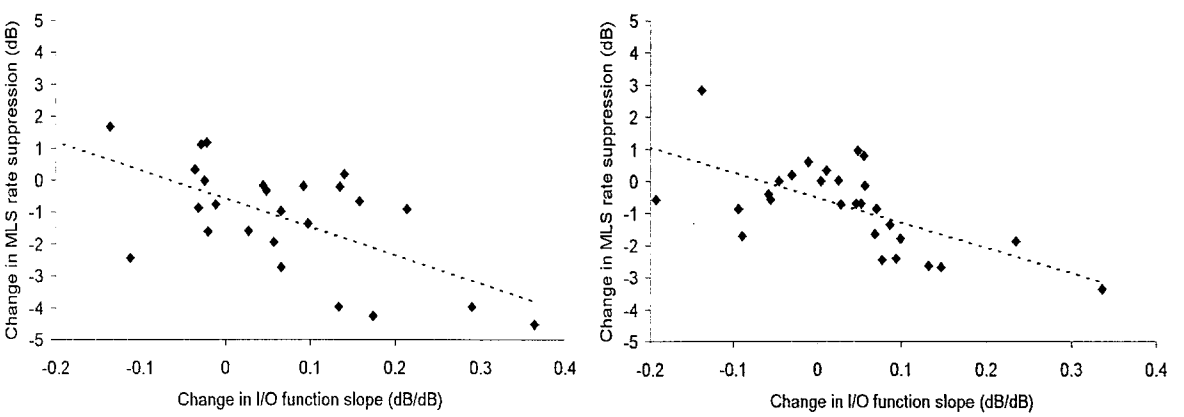


Figure 6-46: A) Change in MLS TEOAE I/O function slope at 2 kHz (50 clicks/s) plotted against the change in MLS TEOAE rate suppression at 2 kHz (70 dB). B) Change in MLS TEOAE I/O function slope for broadband responses (5000 clicks/s) plotted against the change in MLS TEOAE rate suppression for broadband responses (80 dB). Linear regression line plotted.

As the mean change in I/O function slope was so small, the data from all seven sessions at each frequency were combined. These are shown in Figure 6-47. Table 6-20 summarises the relationship between I/O function slope and rate suppression.

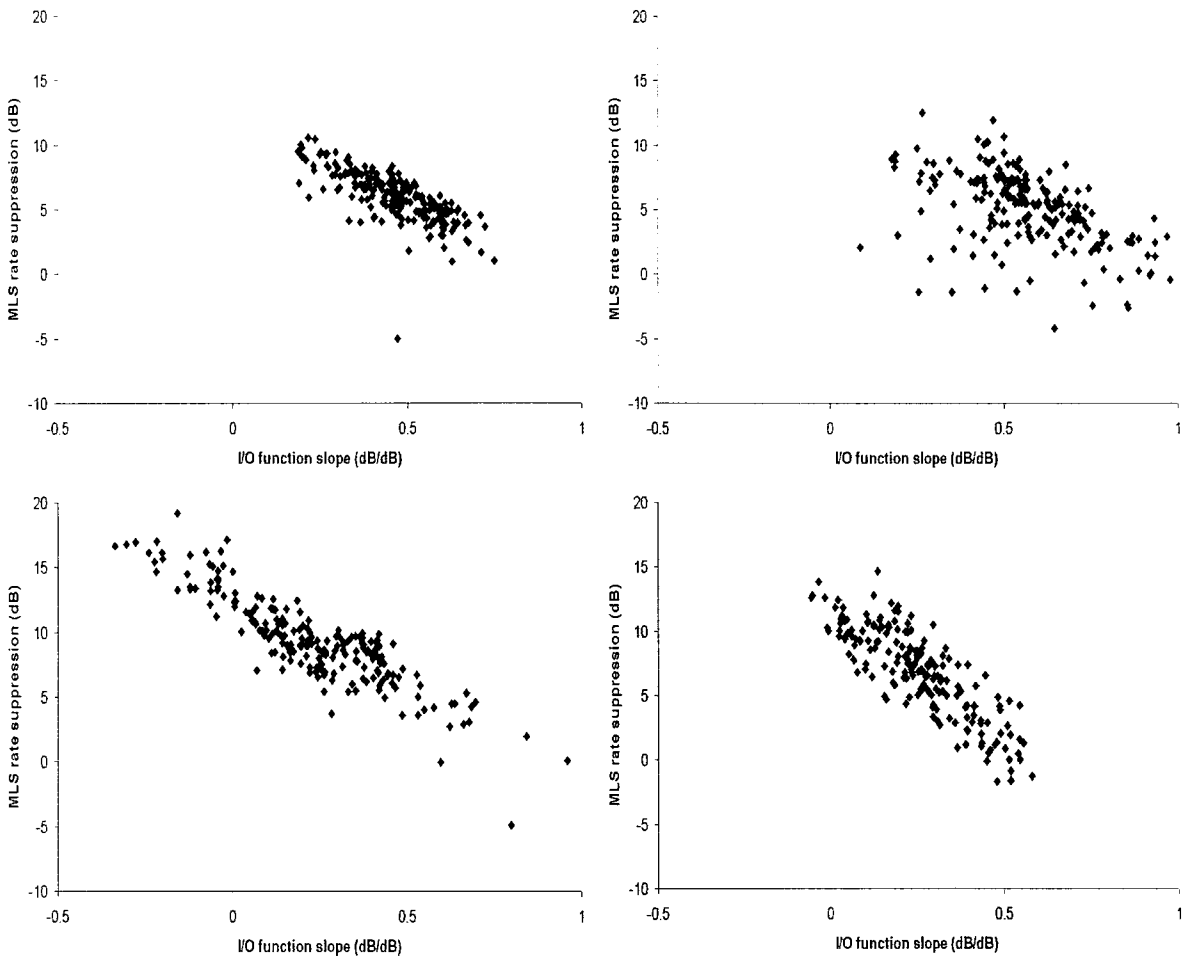


Figure 6-47: Relationship between MLS TEOAE I/O function slope (5000 clicks/s) and MLS rate suppression (80 dB). A) Broadband responses. B) 1 kHz. C) 2 kHz. D) 3 kHz. Data combined from seven sessions and left/right ears.

This shows a high correlation between MLS TEOAE I/O function slope and MLS rate suppression, which improves with increasing frequency. The results at 1 kHz showed wide scatter and variability, but the results at frequencies of 2 and 3 kHz showed a high correlation between I/O function slope and rate suppression. The rate of change of rate suppression with a change in I/O function slope also increases with increasing frequency.

Table 6-20: Results of the correlation analysis of MLS rate suppression (S_{5000}) and MLS TEOAE I/O function slope (5000 clicks/s)

Frequency (kHz)	Correlation coefficient	Slope (dB/dB)
BB	–0.75***	–11.8
1	–0.52***	–8.2
2	–0.89***	–13.7
3	–0.84***	–19.5

*Key: *P≤0.05, ** P≤0.01, *** P≤ 0.005. BB broadband.*

6.6.8.2 MLS rate suppression and HTL

Group analysis

It was predicted from the model that changes in rate suppression were related to changes HTL. Changes in rate suppression and HTL were analysed. Changes were calculated by comparing the pre-aspirin results (mean of sessions 1 and 2) with those from session 5 for each subject.

To assess whether group changes in rate suppression were significantly associated with HTL, a correlation analysis was performed. The change in rate suppression was correlated with the change in HTL at each frequency. Variables that were significantly correlated were then further analysed using linear regression (using rate suppression as the independent variable). Significant results are shown in Table 6-21.

This showed low correlations between the change in rate suppression and the change in HTL. For the highest correlations, the change in HTL still only explained 30% of the change in MLS rate suppression. An example of a significant relationship is shown in Figure 6-48.

This shows a general trend of a reduction in rate suppression with an increase in HTL, however there was wide variability in the data. Table 6-21 shows that the relationship between the change in rate suppression and HTL is approximately 1:1 at the lower frequencies, and increases towards 2:1 at the higher frequencies, as shown by the example in Figure 6-48.

Table 6-21: Correlation between the change in MLS rate suppression (independent variable) and the change in HTL (dependent variable)

Rate suppression frequency (kHz)	HTL frequency (kHz)	Click level (dB)	Correlation coefficient (R)	Slope	R-square
BB	1	70	–0.37*	–1.09	0.14
	2	60	–0.49*	–0.64	0.24
	3	70	–0.54***	–2.16	0.29
	4	60	–0.56**	–1.24	0.32
2	1	70	–0.44*	–0.94	0.20
	6	70	–0.44*	–1.54	0.19
3	6	80	–0.53**	–1.64	0.53

*Key: *P≤0.05, ** P≤0.01, *** P≤ 0.005. BB broadband.*

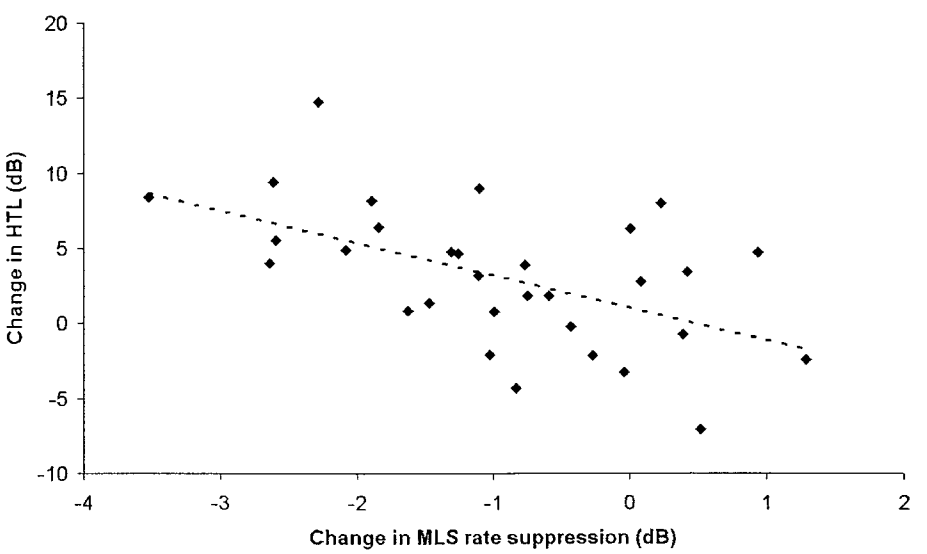


Figure 6-48: Change in MLS TEOAE rate suppression for the broadband response plotted against the change in HTL at 3 kHz. Linear regression line plotted.

Individual subject analysis

The relationship between rate suppression and HTL was then analysed within each individual subject over the seven sessions. Correlation analysis was used to examine the relationship between HTL and MLS TEOAE rate suppression for each subject, ear, frequency and level.

Some subjects showed highly significant correlations between rate suppression and HTL in both the left and right ears for varying combinations of variables. Changes in MLS TEOAE rate suppression and HTL with salicylate consumption were closely related. Correlation coefficient values were 0.7 and above. Most subjects showed highly significant relationships between HTL and at least one MLS TEOAE rate suppression variable. Figure 6-49 shows examples of high correlations between MLS TEOAE rate suppression and HTL for individual subjects.

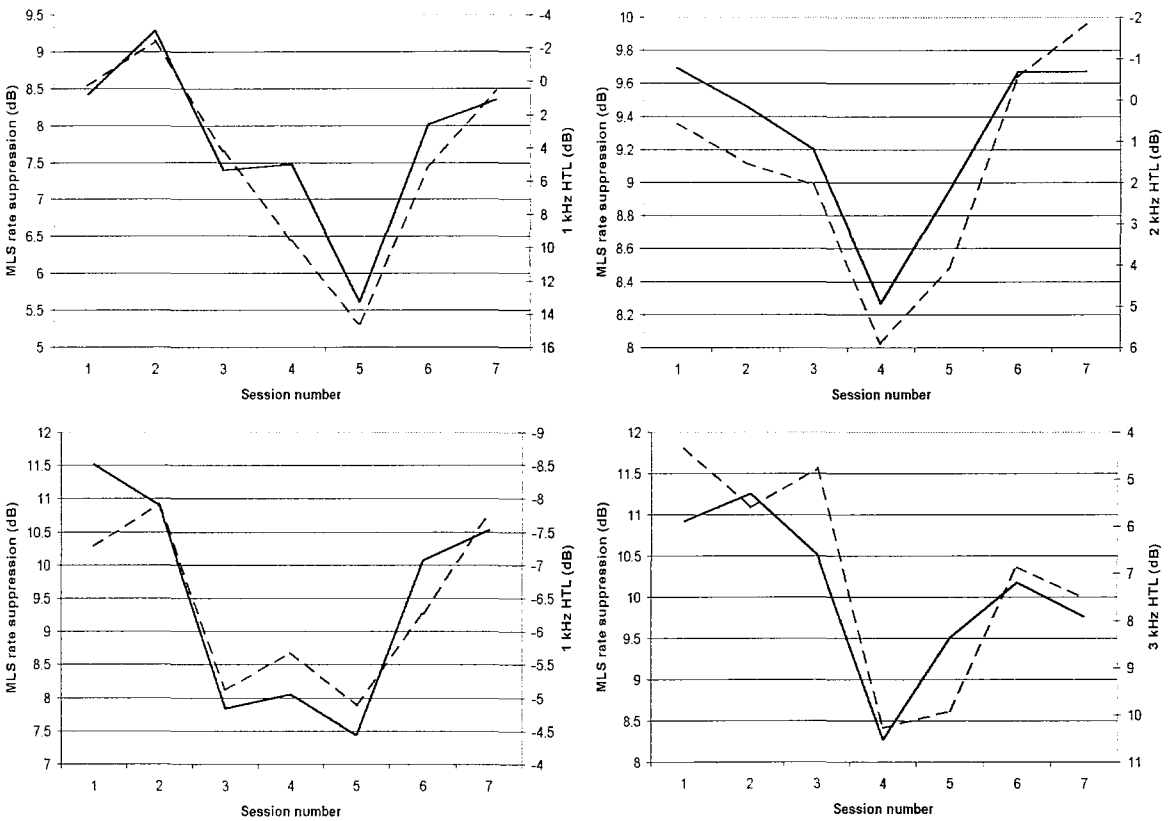


Figure 6-49: Association between MLS TEOAE rate suppression and HTL with salicylate consumption. A) Subject 7, right ear (MLS TEOAE rate suppression broadband response, 70 dB). B) Subject 9, left ear (MLS TEOAE rate suppression, 2 kHz, 80 dB). C) Subject 15, right ear (MLS TEOAE rate suppression 1 kHz, 70 dB). D) Subject 4, left ear (MLS TEOAE rate suppression, 3 kHz, 60 dB). Key – solid line: HTL, dashed line: MLS TEOAE rate suppression.

The number of significant correlations between MLS TEOAE rate suppression and HTL frequencies within subjects and ears was calculated. Five different HTL frequencies were correlated with four different rate suppression frequencies, and the number of significant associations between MLS TEOAE rate suppression and HTL frequencies for each ear was calculated. For each MLS TEOAE rate suppression/HTL frequency combination, a significant association was deemed present if there were significant correlations measured at more than one stimulus level for the rate suppression frequency in question.

Table 6-22 summarises the number of significant relationships between rate suppression and HTL for each subject and ear. There were few significant correlations across subjects and ears. There were also differences between ears subjects in the number of correlations. Figure 6-50 shows the relationship between ears of the number of correlations. This showed more correlations in the right ear than the left ear.

Table 6-22: Summary of the number of significant correlations (P =0.05) between HTL and MLS TEOAE rate suppression

Subject number	Left ear (max 20)	Right ear (max 20)	Left/right total (max 40)
1	0	2	2
2	0	0	0
3	1	0	1
4	0	2	2
6	1	0	1
7	3	6	9
8	1	2	3
9	5	0	5
10	0	0	0
11	0	0	0
12	1	0	1
13	0	0	0
14	1	4	5
15	0	3	3
16	1	0	1
17	0	2	2
19	0	0	0
25^{th} percentile	0	0	0
Median	0	0	1
75^{th} percentile	1	2	2.75

Figure 6-51 summarises the cumulative percentage of subjects according to the number of significant correlations. There was a maximum of 20 possible rate suppression/HTL frequency combinations: therefore using a significance level of 0.05, 1/20 of these were likely to be due to chance. Approximately 30% of ears and 40% of subjects had two or more significant correlations between MLS TEOAE rate suppression and HTL.

The data were examined to determine which MLS TEOAE rate suppression and HTL frequencies were most likely to be significantly related. Figure 6-52 summarises the number of significant correlations between the different variables for all ears. This showed the highest number of correlations between HTL and MLS rate suppression at MLS TEOAE frequencies of 1 and 2 kHz, and for the broadband response. The numbers were approximately equivalent across HTL frequencies of 1-4 kHz. However the number of significant correlations was generally low.

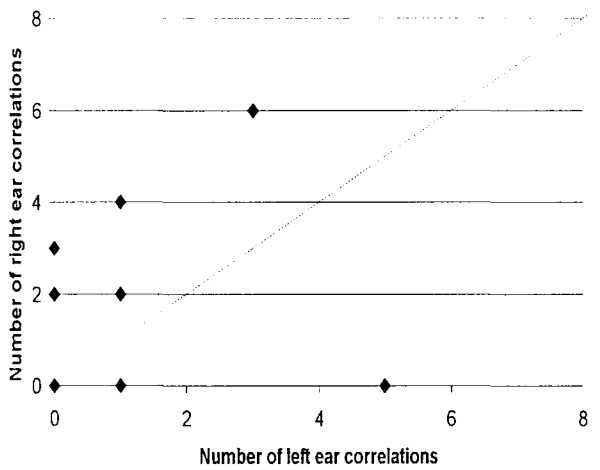


Figure 6-50: Relationship within subjects between the number of significant correlations (MLS TEOAE/HTL) in the left and right ears. The dashed line shows a 1:1 relationship. There was 18% correlation between the number of correlations in the left and right ears.

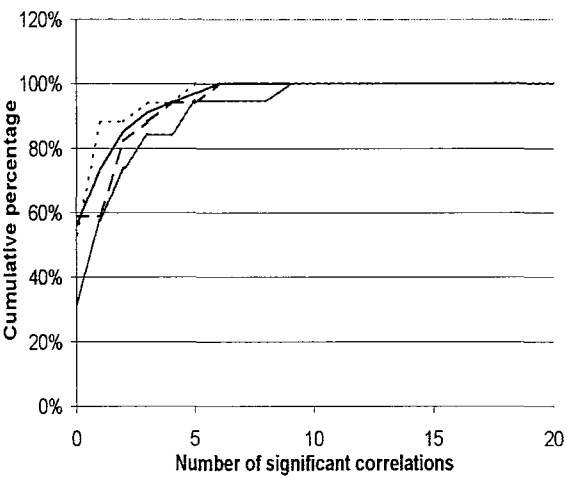


Figure 6-51: Cumulative % of ears/subjects with significant correlations. Key – dark solid line: left/right ears combined, dashed line: right ear, dotted line: left ear, grey solid line: subjects.

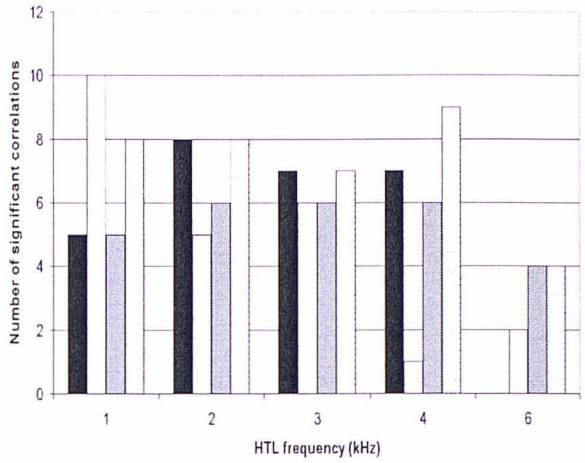


Figure 6-52: Summary of the frequency relationship of the significant correlations between HTL and MLS TEOAE rate suppression frequencies during salicylate consumption. Key - MLS TEOAE rate suppression frequency black bars: 1 kHz, white bars: 2 kHz, grey bars: 3 kHz, stippled bars: broadband response, striped bars.

The data were examined to determine if there were factors that predicted those subjects who showed highly significant relationships between MLS TEOAE rate suppression and HTL changes. Factors examined were: initial starting HTL, size of the change in MLS TEOAE rate suppression, and size of the change in HTL (at individual frequencies and average frequencies). Analysis of these factors showed no marked effect of these on the relationship between MLS TEOAE rate suppression and HTL.

In those subjects with significant relationships between MLS TEOAE rate suppression and HTL, the individual relationships between the variables were examined in further detail. Figure 6-53 illustrates the relationship between the HTL and MLS TEOAE rate suppression for several subjects across sessions. This showed that there are similarities in some subjects in the relationship between the change in MLS TEOAE rate suppression and the change in HTL during salicylate consumption.

The slope relating HTL and MLS TEOAE rate suppression was calculated for each subject using linear regression. MLS TEOAE was used as the independent variable. Figure 6-54 summarises the median slope values relating the MLS TEOAE rate suppression and HTL across the sessions. Points were only included where there were more than two cases. Standard deviation bars are omitted.

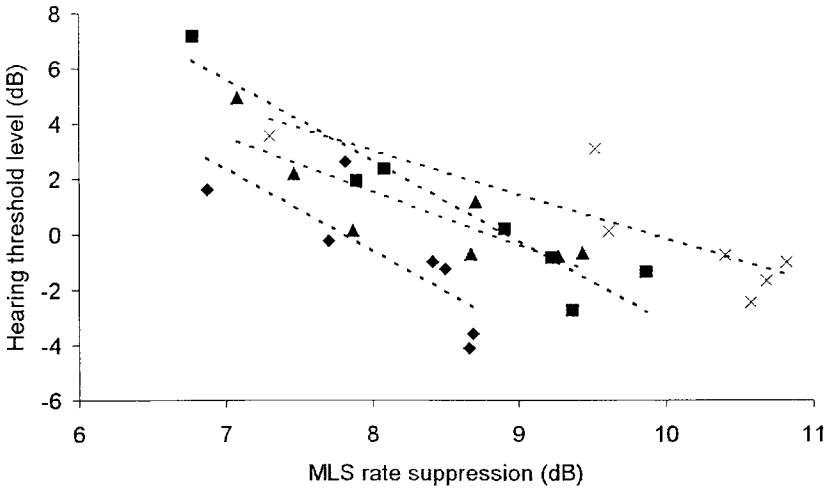


Figure 6-53: HTL at 2 kHz versus MLS TEOAE rate suppression for broadband responses (70 dB) across sessions. Key to symbols - diamonds: subject 3, squares: subject 7, triangles: subject 9, crosses: subject 16.

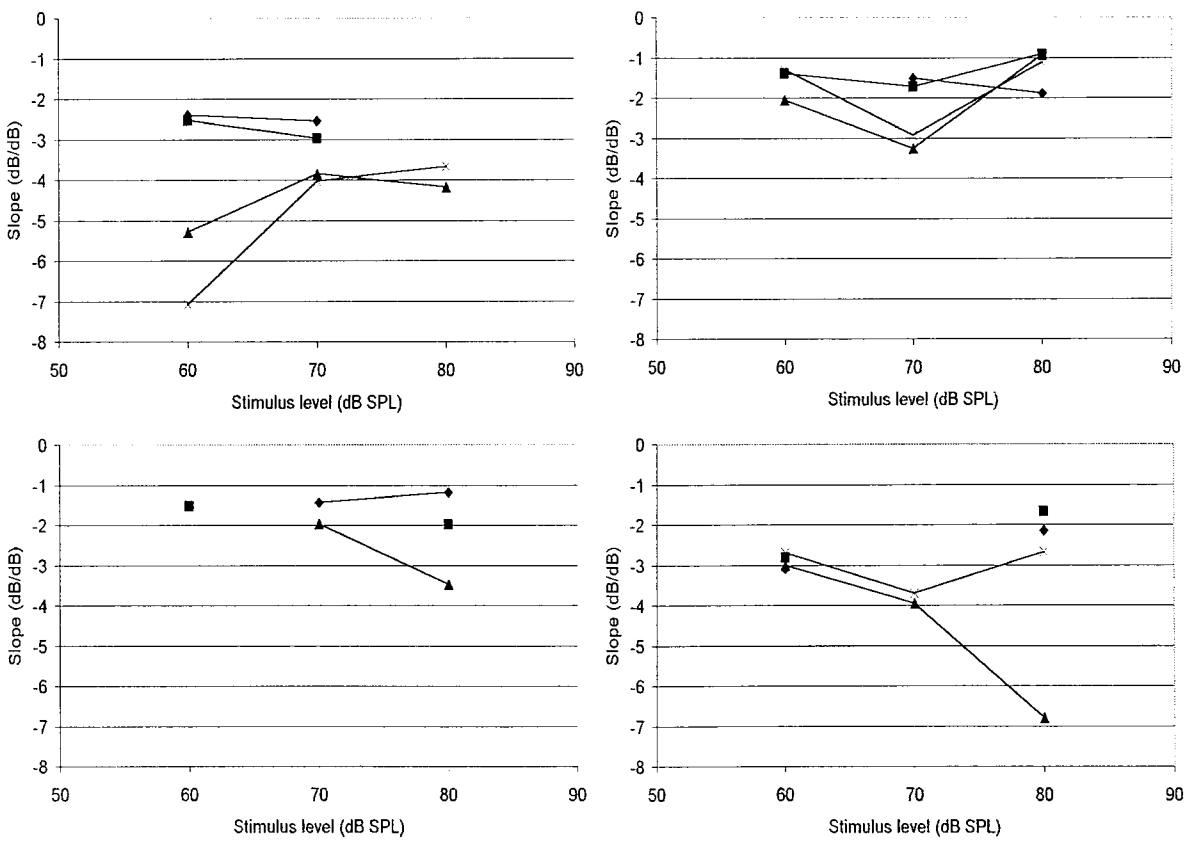
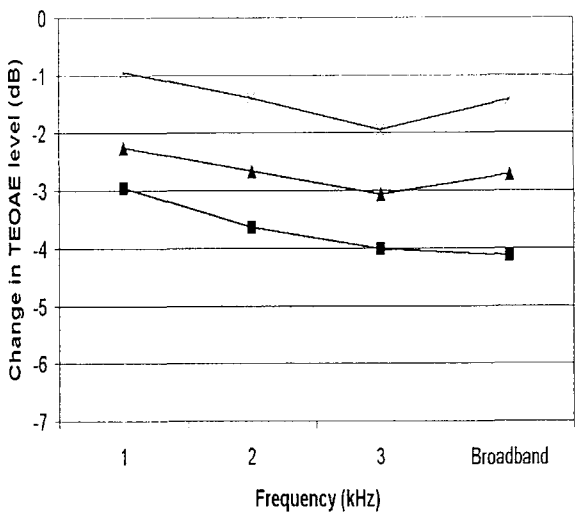


Figure 6-54: Median slope values of the relationship between MLS TEOAE rate suppression (independent variable) and HTL (dependent variable). Graphs are plotted at each MLS TEOAE rate suppression frequency. A) MLS broadband responses. B) MLS 1 kHz. C) MLS 2 kHz. D) MLS 3 kHz. Key to symbols – HTL frequencies, diamonds: 1 kHz, squares: 2 kHz, triangles: 3 kHz, crosses: 4 kHz

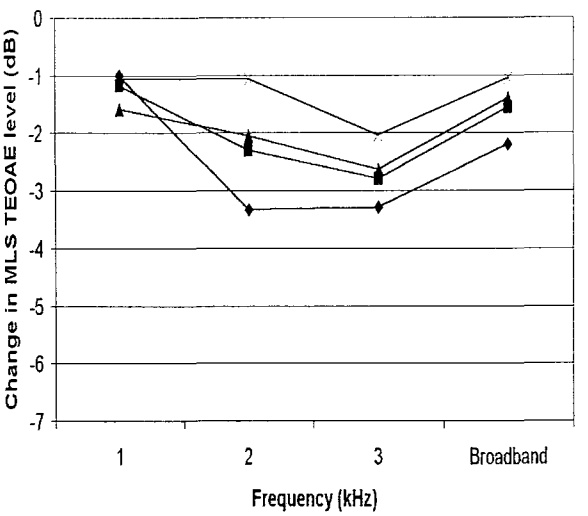
6.6.9 Comparison of DPOAE and TEOAE

6.6.9.1 Comparison of DPOAE and TEOAE level

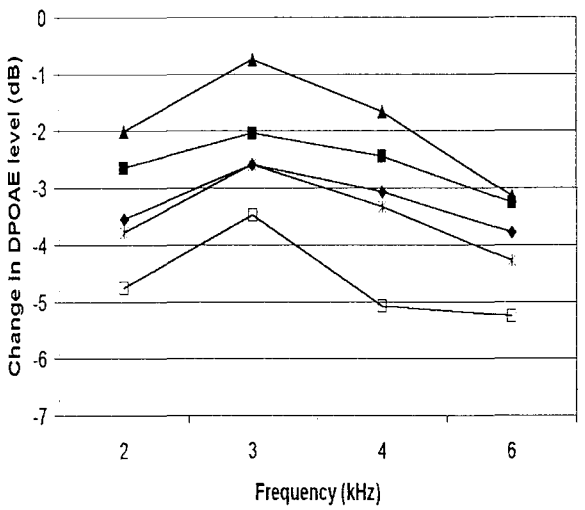
Within-subject comparisons of changes in TE and DPOAE were made to assess the similarities and differences in the effect of salicylate on the two types of OAE. A comparison was made of the changes in level of the two types of OAE, at different stimulus levels. Figure 6-55 compares the mean change across frequency and stimulus level.



A) TEOAE



B) MLS TEOAE



C) DPOAE

Figure 6-55: Mean changes in OAE level with salicylate consumption at session 5 (averaged across left/right ears). A) TEOAE. B) MLS TEOAE. C) DPOAE. Key to symbols - stimulus level crosses: 80 dB, triangles: 70 dB, squares: 60 dB, diamonds: 50 dB, open squares: 40 dB, asterisks: 30 dB.

This showed similar mean changes in level for DPOAE and TEOAE for most stimulus intensity levels. As stimulus level decreased, the change in OAE level increased. The largest change in level occurred for DPOAE evoked by a stimulus level of 40 dB. This was actually greater than the change

at 30 dB, probably because many of the responses at this lowest level were close to the noise floor, and therefore showed no change with salicylate.

For TEOAE, there was an increase in the change in level with increasing frequency. Responses at 3 kHz for both conventional and MLS recording generally showed the largest changes. For DPOAE, from 3 to 6 kHz there was also an increase in level change with increasing frequency. However the changes at 2 kHz were on average larger than the changes at 3 kHz.

The change in TEOAE (both conventional and MLS recording techniques) and DPOAE was calculated for each subject by subtracting the OAE level measured at session 5 from the mean level of sessions 1 and 2. The relationship between these changes in OAE was assessed using correlation coefficient analysis across frequency and stimulus level. The significant results of these analyses are summarised in Figure 6-56 and Figure 6-57.

This showed only weakly significant correlations between the change in DP and the change in TEOAE level. Relationships were significant between DPOAE at 3 and 4 kHz and TEOAE recorded at most frequencies. Most significant correlations were measured between DPOAE evoked at stimulus levels between 40 to 60 dB and TEOAE evoked at 70 and 80 dB. The frequency relationship between DPOAE and TEOAE recorded conventionally was very similar to the relationship between DPOAE and MLS TEOAE.

The next step was to assess the relationship between DP and TEOAE level under the conditions pre-salicylate and peri-salicylate to assess whether salicylate had an effect on the association between the two OAE types.

Correlation analysis and linear regression were performed examining the relationship between TEOAE and DPOAE level pre-salicylate (session 1) and peri-salicylate (session 5). Stimulus levels chosen for investigation were the levels that showed the highest correlations between the two OAE types was with TEOAE evoked by 70 dB stimuli (except the broadband response at 60 dB) and DPOAE evoked by 60 dB L2 stimuli. All frequencies were examined.

Table 6-23 shows the results of the linear regression analysis under the two conditions: pre-salicylate versus peri-salicylate. This showed that for some combination of frequencies there was a marked difference between the correlation of DP and TEOAE in the pre-salicylate and the peri-salicylate conditions. For some frequencies, there was an increase in the correlation of TEOAE with DPOAE from the pre- to the post-salicylate condition. This only occurred at TEOAE frequencies that were below the DPOAE frequency and also for the broadband TEOAE response. There was no change in correlation between DP and TEOAE level when the TEOAE frequency was the same or greater than the DPOAE frequency. Example scattergrams showing the improvement of the relationship between DP and TEOAE are shown in Figure 6-59.

The most striking example is the correlation between TEOAE at 1 kHz, and DPOAE at 6 kHz. Pre-salicylate there was no significant correlation between the two OAE types. However at session 5, the correlation coefficient was 0.55. The scattergram in Figure 6-59 indicates that this improvement in the relationship is a result of a reduction in DPOAE level compared to TEOAE.

Table 6-23: Results of correlation analysis comparing the relationship of TEOAE and DPOAE level pre-salicylate and peri-salicylate

DPOAE Frequency (kHz)	TEOAE Frequency (kHz)	Pre-salicylate (session 1)			Peri-salicylate (session 5)		
		CC	Slope (dB/dB)	R-square	CC	Slope (dB/dB)	R-square
2	BB	0.68	1.02	0.46	0.64	1.18	0.40
	1	**0.26**	**0.41**	**0.07**	**0.40**	**0.77**	**0.16**
	2	0.61	0.62	0.38	0.56	1.01	0.31
	3	0.43	0.91	0.18	0.25	0.72	0.06
3	BB	**0.55**	**0.83**	**0.30**	**0.66**	**0.95**	**0.45**
	1	**0.30**	**0.48**	**0.09**	**0.50**	**0.75**	**0.25**
	2	0.58	0.73	0.34	0.60	0.80	0.36
	3	0.31	0.70	0.09	0.38	0.75	0.14
4	BB	**0.49**	**0.61**	**0.24**	**0.65**	**0.68**	**0.43**
	1	**0.16**	**0.21**	**0.02**	**0.59**	**0.66**	**0.34**
	2	**0.39**	**0.39**	**0.15**	**0.51**	**0.53**	**0.26**
	3	0.49	0.89	0.24	0.44	0.60	0.20
6	BB	**0.46**	**0.60**	**0.04**	**0.52**	**0.82**	**0.27**
	1	**0.02**	**0.00**	**0.00**	**0.55**	**0.96**	**0.30**
	2	**0.23**	**0.20**	**0.05**	**0.43**	**0.71**	**0.18**
	3	**0.41**	**0.89**	**0.16**	**0.62**	**1.08**	**0.38**

Note: Figures in bold show the variables where there was an improvement in correlation coefficient ≥0.1 peri-salicylate. Key to abbreviations - BB: broadband response, CC: correlation coefficient.

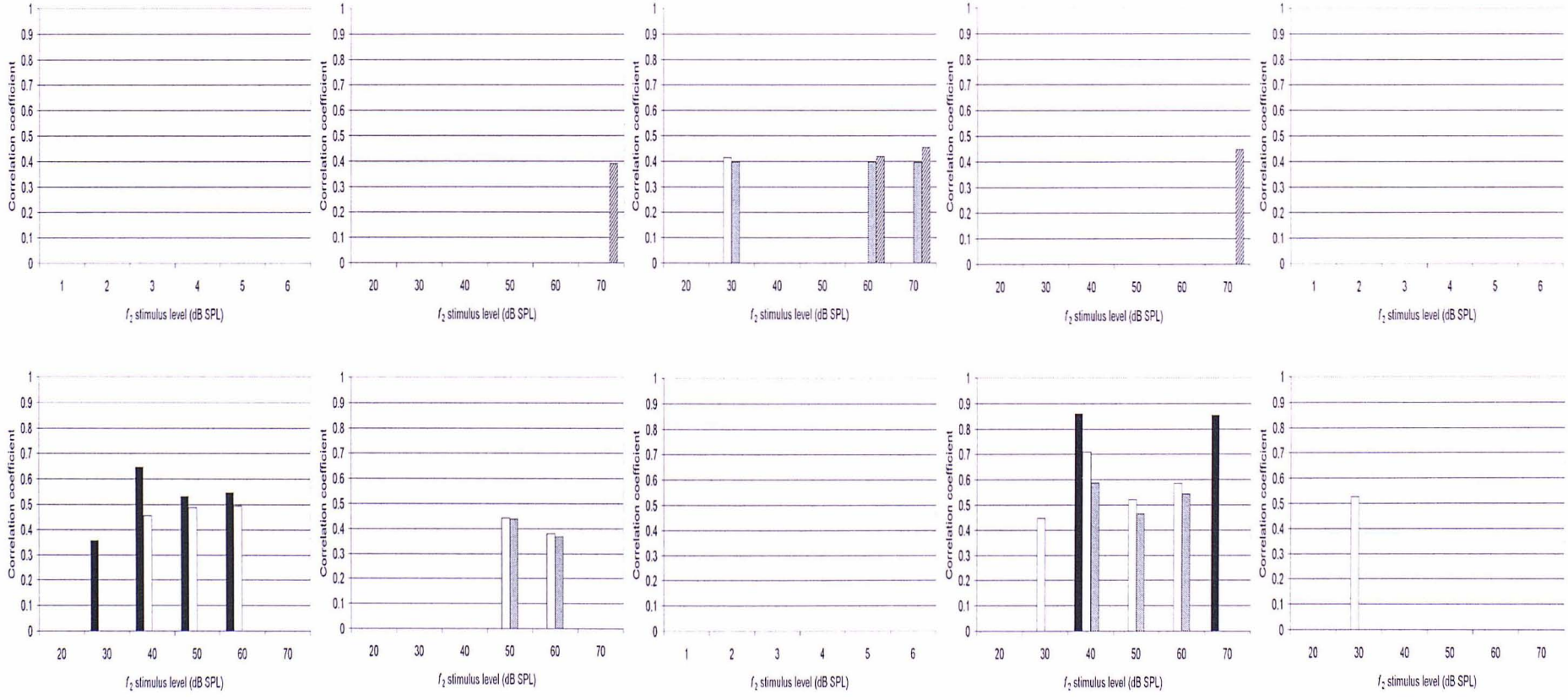


Figure 6-56: Significant correlation coefficients relating the change in TEOAE with the change in DPOAE level following aspirin consumption. Changes in DPOAE at 3 kHz were most highly correlated to changes in the broadband TEOAE, and TEOAE at 3 kHz. Key to bars: TEOAE stimulus level – black: 60 dB, white: 70 dB, grey: 80 dB, striped: 90 dB.

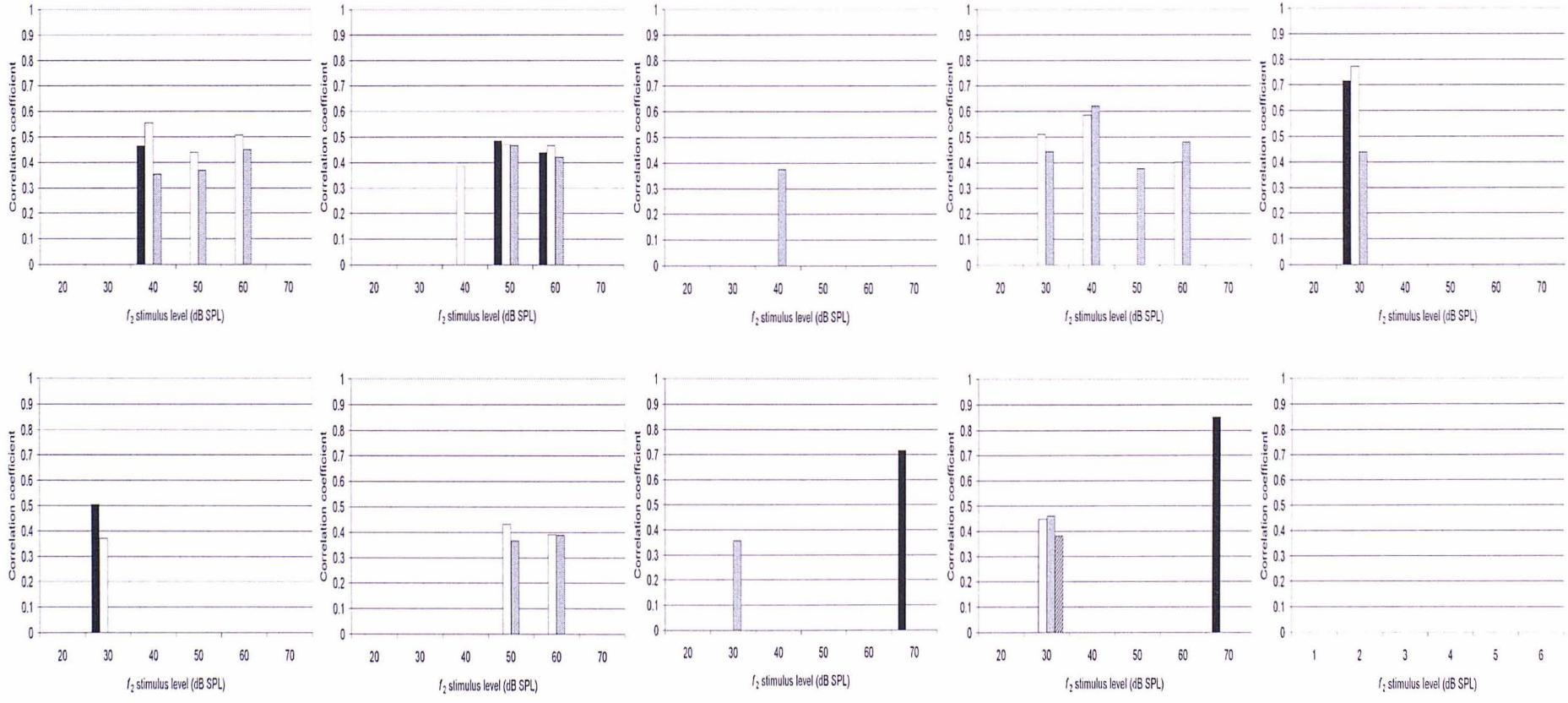


Figure 6-56 cont'd: Significant correlation coefficients relating the change in TEOAE with the change in DPOAE level following aspirin consumption. Key to bars: TEOAE stimulus level – black: 60 dB, white: 70 dB, grey: 80 dB, striped: 90 dB.

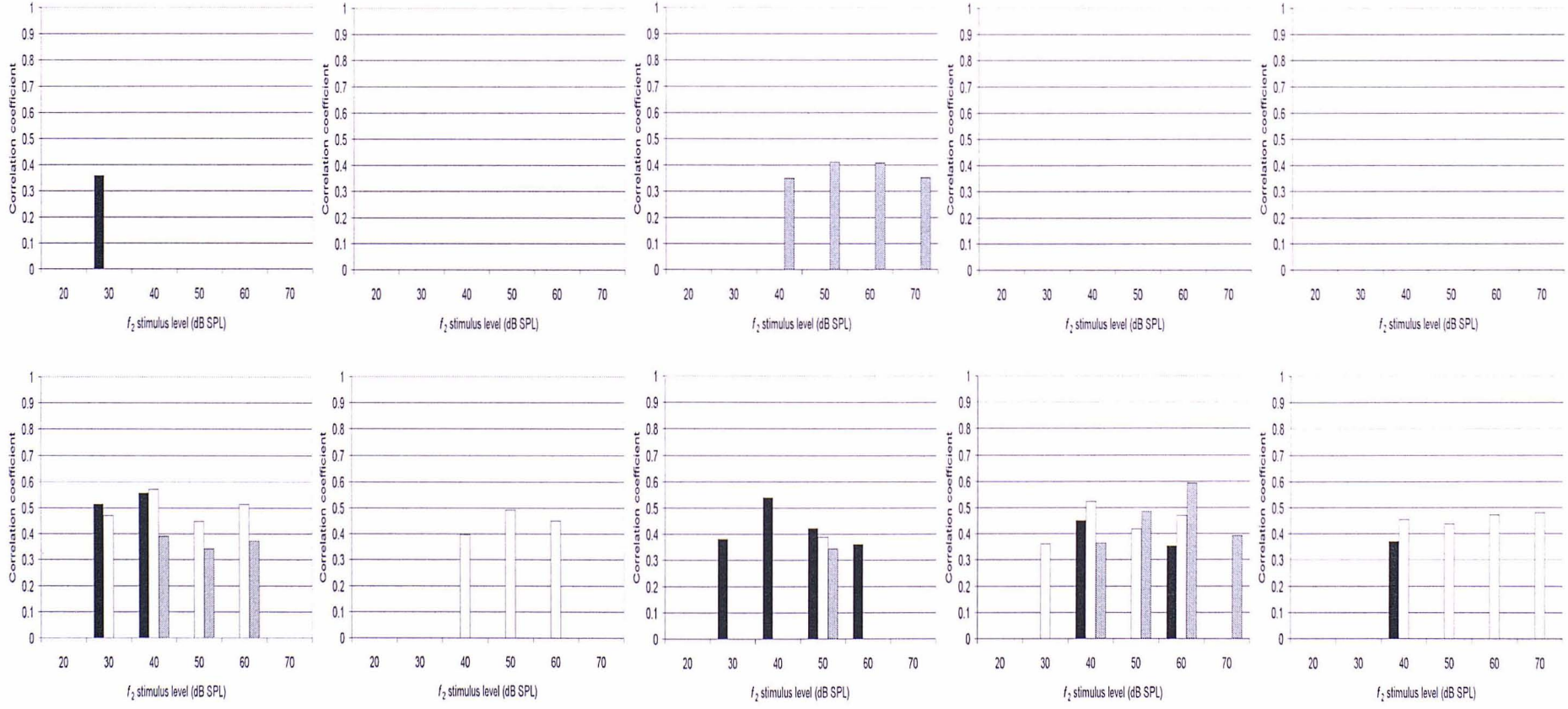


Figure 6-57: Significant correlation coefficients relating the change in MLS TEOAE (5000 clicks/s) with the change in DPOAE level following aspirin consumption. Key to bars: MLS TEOAE stimulus level – black: 50 dB, white: 60 dB, grey: 70 dB, striped: 80 dB.

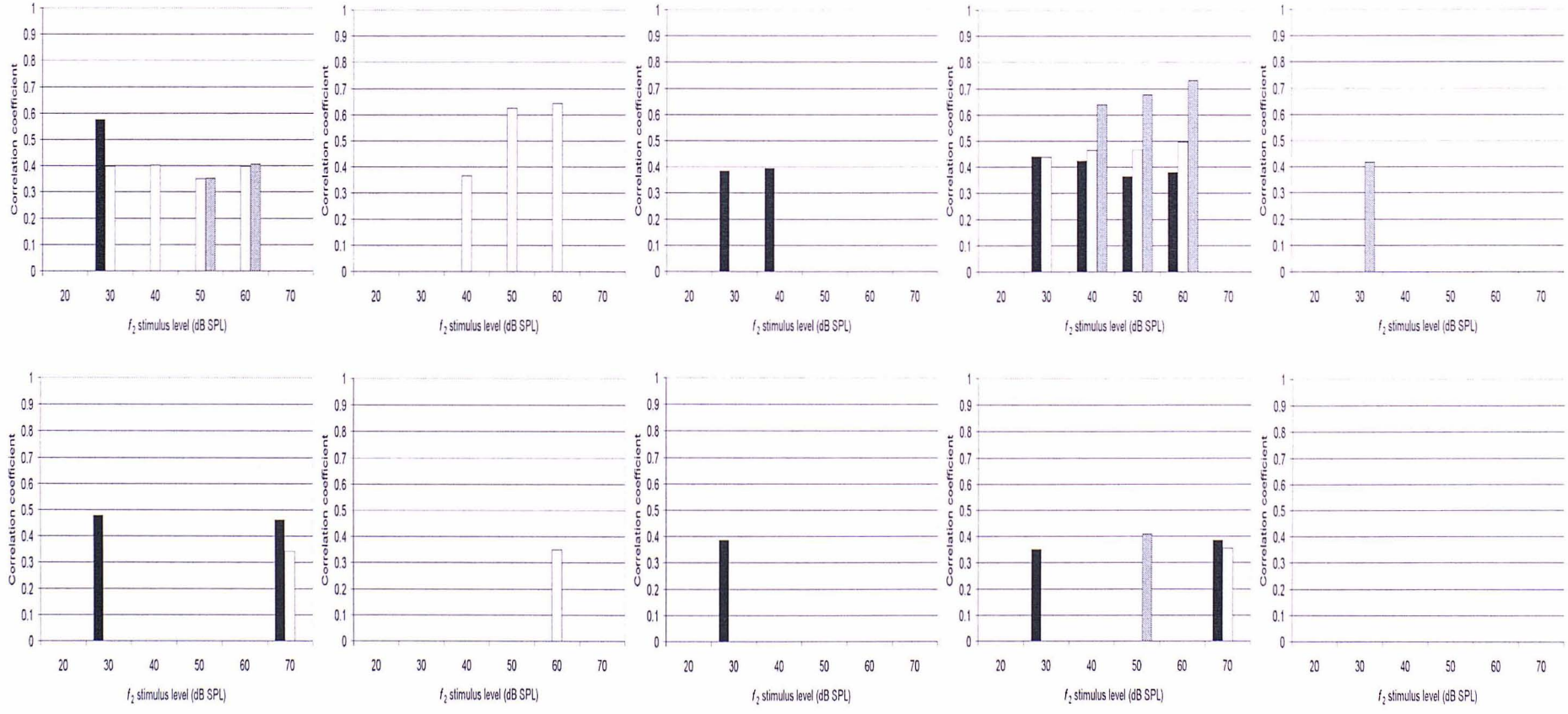


Figure 6-57 cont'd: Significant correlation coefficients relating the change in MLS TEOAE (5000 clicks/s) with the change in DPOAE level following aspirin consumption. Key to bars: MLS TEOAE stimulus level – black: 50 dB, white: 60 dB, grey: 70 dB, striped: 80 dB.

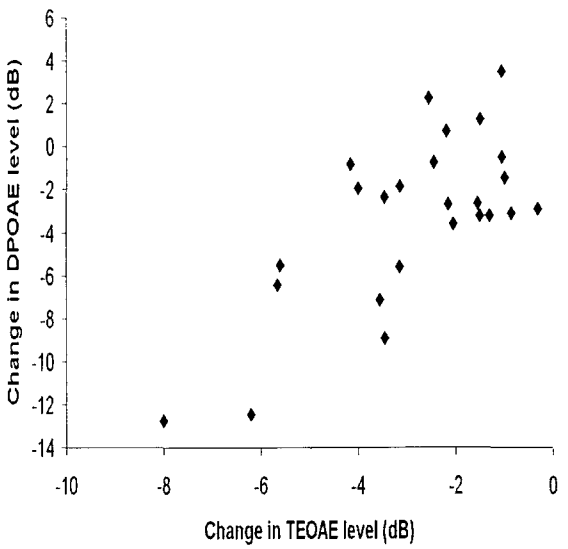


Figure 6-58: Scattergram of the change in DPOAE level versus the change in TEOAE level (3kHz, 70 dB).

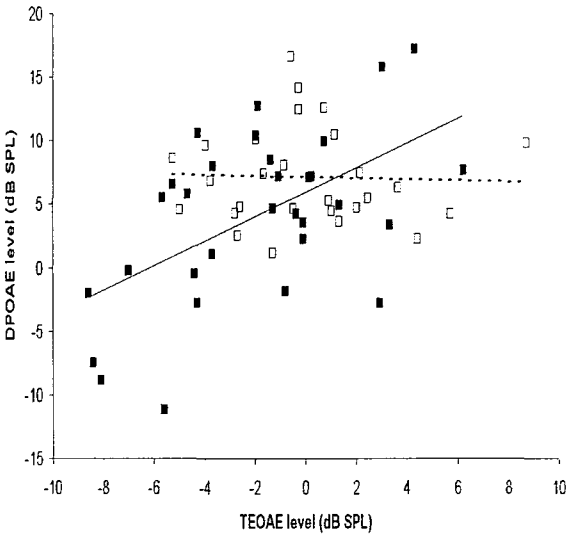


Figure 6-59: Scattergram of TEOAE level at 1 kHz (70 dB) versus DPOAE level at 6 kHz (60 dB). Key to symbols – open squares: session 1, filled squares: session 5. Linear regression lines shown, dashed line: session 1, solid line: session 5.

6.6.9.2 Relationship between TEOAE, DPOAE and HTL

The relationship between the change in DP and TEOAE across sessions was further analysed. This was to determine whether changes in DP and TEOAE occurred concurrently, or whether there were some subjects that showed changes in DP and not TEOAE, and vice versa. The relationship of these changes with HTL at the same frequency was also examined.

DP and TEOAE were compared at frequencies of 2 and 3 kHz, as these were the frequencies at which there were data for both OAE types. OAE were compared at each frequency and the changes with salicylate examined. Stimulus levels examined were those that were sensitive to salicylate i.e. low-level stimulus intensities, but at which responses could be recorded above the noise floor at each session. TEOAE were therefore examined at 70 dB, and DPOAE at 40 dB. These stimulus levels also gave OAE responses of approximately similar levels.

Analysis of the individual subject data showed three types of relationship between DP, TEOAE and HTL at the chosen frequencies. Listed in order of prevalence, these were:

- DP, TEOAE and HTL all showed related changes with salicylate
- DP and TEOAE level showed related changes with salicylate, but no change in HTL
- No change in DP, TEOAE or HTL with salicylate.

There was one example of a change in TEOAE, with no change in DPOAE or HTL. Examples of these different relationships are shown in Figure 6-60.

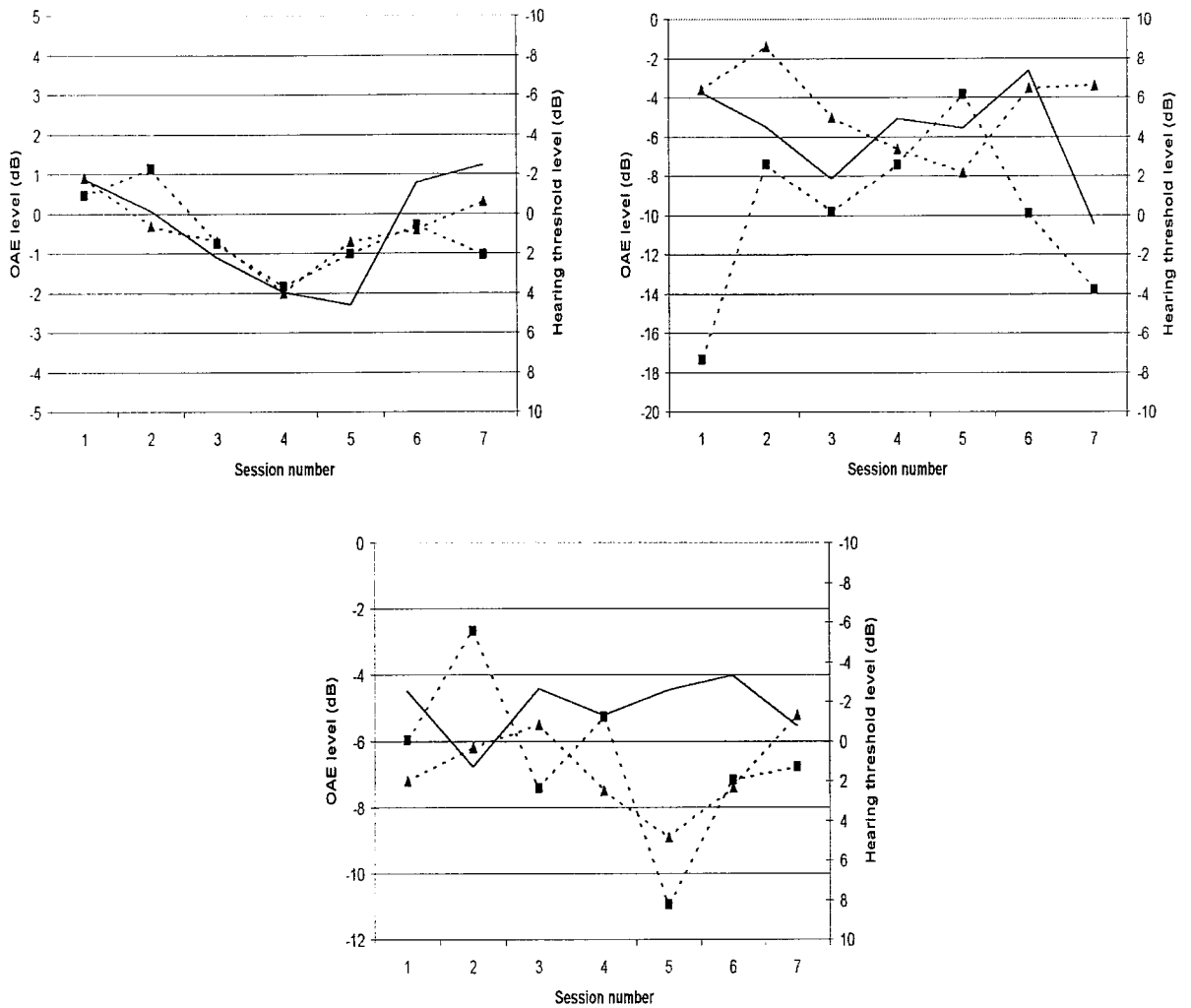


Figure 6-60: Examples of the different types of relationship between DP, TEOAE and HTL. A) Subject 8 (3 kHz) shows a reduction in level of both DP and TEOAE. There is also an increase in hearing threshold at the same frequency. B) Subject 3 (2 kHz) shows a reduction in TEOAE level, but no consistent change in either DPOAE level or HTL. C) Subject 4 (2 kHz) shows a reduction in DP and TEOAE level with no significant change in HTL. Key - solid line: HTL, dashed line/triangles: TEOAE, dashed line/squares: DPOAE.

The most common relationship was for both OAE types and HTL to show similar changes. However there was no consistency across subjects and it was possible for one ear of a subject to show one type of relationship, and for the other ear to show a different relationship. This implies the relationship between DP and TEOAE is very variable both between and within subjects.

Although there were similarities in the reduction of OAE level between TE and DPOAE, the relationship of the two OAE types with HTL were different. Another method of examining the relationship between DP and TEOAE was to examine the frequencies at which there were significant correlations with HTL and to compare for any similarities. Graphs were plotted of the frequencies at which there were significant correlations between the change in OAE and the change in HTL for each subject. This showed no similarities between the two OAE types and the frequencies that were significantly related to HTL.

The data were examined to determine whether subjects were more likely to have significant relationships between HTL and DPOAE or TEOAE, or both. This was to determine whether TEOAE or DPOAE were more likely to be related to HTL, and whether this was a general property of the cochlea, or something specific to individual subjects. Subjects were divided into three groups: whether they showed higher number of correlations between TEOAE and HTL than DPOAE and HTL, a higher number of correlations between DPOAE and HTL than TEOAE and HTL or a similar number of correlations between TEOAE and HTL, and DPOAE and HTL. This showed that most subjects had a higher number of significant correlations between DPOAE and HTL.

6.7 DISCUSSION

6.7.1 Aspirin-induced hearing loss

Salicylate was measured in the blood samples of all subjects. All subjects were compliant with the aspirin regime. There was no relationship between total plasma salicylate and HTL shift. This is consistent with Day et al (1989) who found no significant relationship between HTL shift and total plasma salicylate when examined over the concentration achieved in this study. Day et al (1989) showed a significant linear relationship between unbound plasma salicylate and HTL shift, however unbound plasma salicylate was not measured in this study so this could not be assessed.

There was variation between subjects and also between ears in the effect of salicylate on hearing. This is consistent with other studies investigating the effect of aspirin on hearing that showed a wide range of aspirin-induced hearing threshold shifts: Brown et al (1993) report the audiograms of four subjects during aspirin consumption, which shows variation across frequency, and across subjects in the degree of hearing loss. Carylon and Butt (1993) report similar variation across and within subjects. However in the six subjects tested by Hicks and Bacon (1999), they report varying degrees of aspirin induced threshold shift across subjects, although the shifts within subjects across frequency are generally flat. The inter-subject difference is likely to be related to individual differences in aspirin susceptibility, which may be related to different protein concentration in the blood available to bind salicylate. Subjects with higher protein concentration can bind more salicylate leaving less unbound

salicylate available in the blood to have an ototoxic effect. It may also be related to inherent genetic or physiological differences between subjects. There are known differences between subjects in their susceptibility to factors such as noise on hearing for example melanin (Barrenas and Lindgren, 1990; 1991). Such factors may also be responsible for differences in susceptibility to aspirin. Reasons for the differences between ears recorded in this thesis is unclear.

Although most subjects received a temporary hearing threshold shift from aspirin, no single subject obtained a hearing loss of 20 dB HL or greater at any frequency. This is likely to be a limitation of the aspirin dose regime. If it had been possible to give a higher dosage of aspirin, greater temporary hearing shifts are likely to have been obtained. Myers and Bernstein (1965) obtained hearing threshold shifts of up to 40 dB with daily aspirin dosages up to 8g. This dosage is more than double the daily concentration used in the present study.

In the present study, there was no differential effect of salicylate at any particular audiometric frequency. Unlike McCabe and Dey (1965) who showed a greater effect of aspirin at the higher frequencies, in the present study all frequencies were similarly affected. The difference may be due to the higher aspirin dosage used in their study, which was approximately 2 g greater.

The main problem with the experimental design was the limitation of the size of the temporary hearing threshold shift that could be induced. Karlsson and Berninger (1995) obtained a temporary hearing loss in one subject of 46 dB using quinine, but the methodology used is unsuitable for studies of volunteers. Unlike aspirin, quinine can induce permanent hearing threshold shifts in some subjects and for this reason was not considered a suitable method. Noise has also been used to generate TTS, and hearing threshold shifts of approximately 40 dB have been recorded. However it is unethical to maintain noise-induced TTS for three days as required in this experiment, as this could result in permanent hearing threshold shift.

6.7.2 DPOAE

Salicylate had a significant effect on DPOAE level. The changes in DPOAE level were related to the evoking stimulus intensity level. There was little or no reduction in DPOAE level at the highest stimulus levels with the largest reduction occurring at the lower stimulus levels across all frequencies. The biggest changes occurred at an L2 level of 30 dB at all frequencies. This is consistent with other studies that suggest lower intensity stimulus levels are most sensitive to changes in cochlear function (Brown et al, 1996). Previous experiments on salicylate and DPOAE have shown similar results. Salicylate has caused a reduction in the $2f_1-f_2$ DPOAE level (Brown et al, 1993). This reduction was most marked at the lower intensity stimulus levels (Wier et al, 1988). A study on gerbils showed a larger reduction in the $2f_1-f_2$ DP level, with changes between 5 and 30 dB (Frank and Kössl, 1996). Interestingly, there was also an initial increase in the level of the f_2-f_1 DPOAE, which subsequently decreased. This was thought to be due to a change in the operating point of the cochlear amplifier.

This differential change in low compared to high intensity DPOAE level in this experiment gave an overall mean change in I/O function shape showing a reduction in compression and therefore altered cochlear nonlinearity. All frequencies showed a reduction in nonlinearity with the smallest change measured at 6 kHz. These changes in I/O functions are consistent with a reduction in cochlear amplification mechanism. There was large variation between individuals in the changes in I/O functions. Some subjects showed considerable changes whereas others showed no marked change. It was not possible to determine the reason for these differences between subjects.

The results obtained from this experiment were compared to the framework, based on Mills' model of DPOAE I/O functions. The framework predicted with increasing HTL a reduction in DPOAE level greatest at lower stimulus level and a reduction in compression. The results were consistent to a degree with the framework. The maximum recorded change in DPOAE level in this experiment was 15 dB at stimulus intensity levels of 40/50 dB. The reduction in nonlinearity of the I/O functions was characterised by an increase in slope value. This gave a mean increase in DPOAE growth from 0.79 dB/dB, pre-salicylate to 0.93 dB/dB peri-salicylate. The maximum growth rate recorded peri-salicylate was 1.7 dB/dB in one subject only. None of the functions reached a value of 3 dB/dB, or even 2 dB/dB as recorded by Mills.

Limitations in the dose of salicylate may account for these small changes. It is possible that a higher dose would have induced larger changes in DPOAE and a higher DPOAE growth rate. Ideally aspirin-induced hearing losses of 40 to 60 dB are required, indicating a large reduction in OHC function, however the maximum aspirin-induced hearing loss in this experiment was 20 dB HL. Other studies of human subjects reported similar values to those reported here. Janssen et al (2000) recorded DPOAE from a woman who took an overdose of salicylate, a dose estimated to be 10 g. The woman gained a 50 dB hearing loss at 6 kHz, and during the acute phase of aspirin intoxication, slopes of DPOAE I/O functions at 6 to 8 kHz approached 1.5 dB/dB. On recovery the DPOAE I/O function slopes around these frequencies were 0.5 dB/dB. Dorn et al (2001) measured DPOAE I/O functions in subjects with a range of HTL. The maximum recorded slope values were approximately 2 dB/dB across the frequency range in subjects with severe hearing losses.

The relationship between the changes in DPOAE and HTL was examined. This experiment tested the hypothesis that the moderate relationship reported in the literature between DPOAE and HTL (e.g. Gorga et al, 1993a, b) is a result of inter-subject and inter-ear differences masking the relationship. Longitudinal changes in DPOAE and HTL were therefore expected to have a higher correlation than the cross-sectional differences measured here in Experiment 1.

Examination of the group changes in DPOAE level showed only a low correlation with changes in HTL. The trend occurred in the expected direction, with an increase in HTL associated with a reduction in OAE level. This was observed for all frequencies and overall the 3 kHz DPOAE had the

highest correlation (–0.54) with HTL at 1 kHz. DPOAE evoked by lower stimulus levels had a higher correlation with HTL than those evoked by higher stimulus levels.

The hypothesised higher correlation between the group changes in OAE and HTL was not observed. The correlation coefficient values were actually lower than those obtained for the cross-sectional differences in DPOAE level and HTL, and the hypothesis that longitudinal changes would have a higher correlation than cross-sectional differences was not supported.

The relationship of the change in DPOAE level and HTL was then examined in more detail for individual subjects and ears. For a particular combination of DPOAE and HTL variables, some subjects showed highly significant relationship between the change in DPOAE and HTL, whereas others did not show significant relationships. There were also differences within-subjects: some subjects showed significant relationships between OAE and HTL in one ear but not in the other.

Those ears with significant relationships showed an increase in HTL closely associated with a decrease in DPOAE level. For these ears, the change in DPOAE level occurred concurrently with the change in HTL, and the correlation coefficient values were highly significant and greater than 0.8. This shows a direct relationship between a change in DPOAE level and a change in HTL for some ears, but not all. Approximately 40% of ears showed no significant relationship between DPOAE and HTL. The remaining 60% showed combinations of significant correlations, varying from few to many, across a range of frequencies.

There were differences between subjects and ears as to which HTL frequencies were significantly associated with which DPOAE frequencies. Some ears showed many significant associations between most combinations of DPOAE/HTL variables, whereas others showed fewer associations, or in several cases no relationship at all. In general, the highest correlations between DPOAE and HTL occurred at stimulus levels of 50 or 60 dB, at HTL frequencies of 3 kHz and DPOAE frequencies of 3 or 4 kHz. These results are consistent with studies of noise exposure on DPOAE, which showed the greatest effect of noise on DPOAE level at approximately ½ to 1 octave greater than the frequency of the noise (Engdahl and Kemp, 1996). This is related to the change of the TW peak position with level. However correlations between DPOAE and HTL were not restricted to these frequencies, and some ears showed significant associations between frequencies that were several octaves apart.

The reason for the differences between subjects and ears in their relationship between DPOAE and HTL was not related to the change in HTL, the change in DPOAE or the plasma salicylate concentration. One possible explanation is that over the course of the two-hour test session, there was a change in plasma salicylate concentration. Because of the different measurement times during the session, DPOAE level and HTL may have been differentially affected by the salicylate at the time of measurement and therefore not be well correlated. However the half-life of salicylate is approximately 15 hours and therefore is unlikely to account for the variability shown. An alternative

explanation could be that salicylate had differential effects on the distortion and reflection mechanisms of DPOAE generation between subjects. Further experimental work is required to understand these differences between subjects and ears.

For those ears with significant relationships, the individual associations between DPOAE level and HTL were examined. This showed that the rate of change of DPOAE with HTL was similar in all ears. This implies a direct relationship, which is similar across these ears, between DPOAE and HTL although it must be stressed that this was not true of all ears. The group analysis reported previously included all ears, so it seems likely that those ears with no significant relationship between DPOAE and HTL were masking the significant results of the other ears.

The other DPOAE measure, *DPOAE stimulus level* was also examined. Unlike DPOAE level there was no significant relationship between group changes of DPOAE stimulus level and HTL, but there were highly significant individual within-ear changes. These results were very similar to the DPOAE level results. *DPOAE stimulus level* was highly correlated to changes in HTL for various combinations of variables and subjects/ears. For individual ears, the same combination of DPOAE and HTL frequencies were correlated as for the DPOAE level and HTL, implying that they were both measuring the same cochlear process. Similarly, the most significant relationships occurred between HTL at 2 and 3 kHz and DPOAE at 3 kHz.

The reason why the direct relationship between DPOAE and HTL in some subjects has not previously been demonstrated is likely to be because other experiments, even longitudinal experiments, have looked at group changes. In this experiment, the results of the group changes were in agreement with other studies and showed only a weak or non-significant relationship between DPOAE and HTL. This study is one of few that measured more than two longitudinal points in time. By analysing individual ear data over the seven sessions, significant relationships were identified in this experiment. Other experiments of temporary hearing threshold shifts have measured changes at two time points: pre- and post-exposure (e.g. Engdahl and Kemp, 1996). A similar experiment by Berninger and Gustafsson (2000) used quinine to induce a TTS. They found no correlation between the DPOAE level shift and HTL shift. However they only compared two points: pre-quinine levels and the maximum shift post-quinine. Similar analyses in this experiment also showed no significant correlation. This experiment was only able to show significant relationships between variables by analysis over seven repeated measurements pre-, peri- and post-salicylate exposure.

The method of measuring HTL in this experiment was designed to give an accurate measure of HTL changes. It is possible that previous experiments showing changes in OAE before changes in HTL did not obtain the most accurate measure of HTL.

From these results it appears that although the hypothesis must be rejected based on the results of the whole group, for approximately 60% of ears the hypothesis is upheld. In these ears, for some

DPOAE/HTL frequency combinations, longitudinal changes in DPOAE and HTL have a higher correlation than the cross-sectional differences. Inter-ear differences are therefore implicated as one of the factors that influence the group variation in the relationship between DPOAE and HTL. However other factors are implicated, and must be included in a model that accounts for all subjects.

The framework based on Mills' model of DPOAE I/O functions is therefore not suitable for all subjects and requires modification. One of the main criticisms of Mills' model is that it only considers cochlear amplification at the f_2 site and does not include other sites along the basilar membrane that may be important for DPOAE generation, such as the $2f_1$-f_2 reflection site. As acknowledged previously, Mills' model is based on the outdated concept of active and passive DPOAE generation. Passive DPOAE, thought to be generated in response to high-level stimuli were described as invulnerable to physiological insult. As the active/passive generation of DPOAE has recently been challenged (Mom et al, 2001; Mills, 2002) the invulnerability of high level DPOAE to salicylate was assessed in the human subjects of this experiment. Results at 6 kHz showed that high-level DPOAE were vulnerable to salicylate, and showed a mean reduction of approximately 3 dB over the course with salicylate. This is consistent with the study by Mom et al (2001). Berninger and Gustafsson (2000) also showed a small mean reduction in DPOAE level of 1 dB at 70 dB stimuli level, independent of frequency. This indicates that the framework of the expected change in DPOAE with increasing HTL needs modification.

The framework based on Mills' model does not take account of factors that may affect DPOAE and HTL generation differentially. There may also have been some small cellular changes in the OHC from salicylate, which are enough to reduce DPOAE level without causing a change in HTL. Cochlear amplification is only one of the factors required for DPOAE generation; nonlinearities and roughness are also important (Talmadge et al, 1998). It is possible that there were changes in these other factors without changes in cochlear amplifier gain, thus resulting in a change in DPOAE without a change in HTL. In the literature there have been proposals that changes in OAE reflect early, pre-audiometric damage (e.g. Desai et al, 1999). It is first necessary to define what is meant by pre-audiometric changes. One definition is that significant changes in OAE are detected when there are no significant changes in HTL. The data from this experiment uphold this definition. The test-retest repeatability of OAE is higher than that of pure tone audiometry; therefore significant changes in OAE can be detected more sensitively than changes in HTL. Alternatively changes in OAE may occur before changes in HTL i.e. that there are changes in the cochlea that affect OAE generation but do not initially affect HTL. These may be changes in roughness that affect OAE generation but not HTL. Again this definition is also upheld by the results from this experiment. There is a complicated relationship with frequency, and evidence that generation of OAE at particular frequencies may be affected by other areas of the cochlea. It is also possible that a change to the cochlea that affects the

reflection component of DPOAE may not cause a change in HTL, but will cause a reduction in DPOAE level.

Salicylate presumably had an effect at various places across the BM and was not simply localised to the audiometric frequencies that were measured. As HTL was not measured at a diverse range of frequencies across the BM (and therefore CA function at these frequencies was not measured), it was not known at which other places along the BM salicylate caused an effect. Although the main generation place for DPOAE is the f_2 place, there are also contributions from other places along the BM. Therefore it is possible in subjects that did not show a good relationship between DPOAE and HTL that the changes in DPOAE were not related to changes in CA function at f_2, but to different places along the BM. Alternatively that there were changes in CA gain at f_2 *and* at another place along the BM. If this were the case, there would be limited direct relationship between DPOAE and HTL at the f_2 frequency. It is proposed that future experiments take into account CA function not only at f_2, but also at f_1, $2f_1$–f_2 and other frequencies around this place. The subjects who showed the most correlations between DPOAE and HTL were the subjects who had a consistent 5 dB shift in HTL at 3 or more frequencies. This suggests that in these subjects salicylate had a constant and uniform effect across the BM and that the change in CA function occurred at the expected f_2 place. The frequency relationship between the changes in DPOAE and HTL was not consistent across subjects.

Another factor that may explain the lack of relationship between DPOAE and HTL is the inner hair cells. Although previous studies of salicylate and hearing indicate that the main effect of salicylate is a reduction in cochlear amplification (Cazals, 2000), there is some evidence that it may also cause a reduction in the outer/inner hair cell coupling (Stypulkowski, 1990). The reduction in turgor in the OHC may also have affected the coupling of the OHC to the tectorial membrane. There are also measured effects of salicylate on cochlear blood supply, and on the afferent nerve activity (Cazals, 2000), and these may affect the output of the inner hair cells. If these effects occurred in some of the experimental subjects, or at some frequencies this may have resulted in an increase in HTL without a similar change in the DPOAE measure. This could explain why there were higher correlations between DPOAE and HTL for some ears and not for others.

To summarise, based on the results of the whole group, the hypothesis that longitudinal changes in DPOAE and HTL show a higher correlation than cross-sectional differences must be rejected. Although in some subjects and ears this hypothesis was upheld, the variability in the relationship between DPOAE and HTL means that overall it must be rejected. The high correlations between combinations of DPOAE and HTL for approximately 60% of ears showed that the underlying relationship between OAE and HTL was upheld for a proportion of ears, and that it was a reasonable hypothesis to make. A relationship between DPOAE and HTL, via the OHC and the CA would therefore appear to be a reasonable basis for a model but other factors must be included and more work is required to develop a model suitable for all ears.

6.7.3 TEOAE

Discussion is limited to specific points regarding TEOAE. Much of the DPOAE discussion also applies to TEOAE.

Salicylate had a significant effect on TEOAE level. The largest changes occurred at the lowest evoking click stimulus intensities. A stimulus intensity of 60 dB was the lowest click intensity at which responses were consistently recorded throughout the salicylate regime, and for which the level of the TEOAE responses was not below the noise floor. The maximum change in TEOAE level with salicylate was 10 dB. This is similar to reports of other studies of human subjects (e.g. Long and Tubis, 1988; Quaranta et al, 1999).

During the period of salicylate ingestion, the conventional TEOAE I/O functions for the broadband response, 1 and 2 kHz showed reductions in level that were greater for lower stimulus levels. At 3 kHz, the level reductions were approximately equal for all stimulus levels, giving a general downward shift in I/O functions at this frequency. Interestingly, the high stimuli intensity TEOAE, particularly at the higher frequencies were sensitive to salicylate. Many subjects showed a reduction in TEOAE level at a click level of 80 dB. This may be related to the spread of excitation of high stimulus click levels along the cochlea.

The change in linearity of TEOAE growth with stimulus level was assessed by measuring the slope of the I/O function and compared to the framework of I/O functions. The framework predicts an increase in linearity of the I/O functions, and a reduction in TEOAE level greatest at low stimulus levels. There was a mean increase in TEOAE growth from 0.44 dB/dB pre-salicylate to 0.58 dB/dB peri-salicylate. The maximum recorded slope value post-salicylate was 0.67, and this was the closest value to the predicted slope of unity. There was variability between subjects in the effect of salicylate on the I/O function, with some subjects showing large reductions in nonlinearity and others showing no change. There were also more changes at the low frequencies than at the high frequencies. The results at the low frequencies were consistent with the proposed framework of TEOAE I/O, with a reduction in nonlinearity, and little or no change in level at the high intensity levels.

The results of the higher frequency TEOAE I/O functions were inconsistent with the proposed framework. At these frequencies, high intensity TEOAE were sensitive to salicylate. This could imply several things. Firstly that the CA had not reached saturation at these high stimulus levels, and therefore was sensitive to salicylate, or secondly that the TEOAE generation mechanism at high intensity levels is sensitive to salicylate contrary to expected. Experimental studies have shown differences in the basal and apical properties of the BM (Robles and Ruggero, 2001). The BM is less finely tuned at the apex than at the base (Cooper and Rhode, 1997). Comparison of the sensitivity in the chinchilla cochlea to low and high stimuli showed a difference of 56 dB at the base, and only 15 dB at the apex. There is also less compressive nonlinearity at the apex. The difference between the

basal and apical BM in human subjects has not been established, however these animal studies imply that CA gain in humans may be higher at the base than at the apex. If this is the case, then the CA will saturate at higher stimulus levels for high compared to low frequencies.

The relationship between the changes in TEOAE and HTL was examined. This experiment tested the hypothesis that the moderate relationship between TEOAE and HTL reported in the literature is a result of inter-subject and inter-ear differences masking the relationship. Longitudinal changes in TEOAE and HTL were therefore expected to have a higher correlation than cross-sectional differences. Following the proposed framework, the change in TEOAE was estimated using two different methods: measuring the change in TEOAE level at a constant stimulus level and measuring the change in stimulus intensity at a constant TEOAE level. The group changes in TEOAE level showed only a low correlation with changes in HTL. Of the analyses based on more than ten data points, a maximum correlation of –0.50 was measured. This was lower than many of the cross-sectional studies reported in the literature (e.g. Sückfull et al, 1996). This result should be compared with those of Berninger et al (1998) who showed a significant relationship between the change in HTL and TEOAE detection threshold from quinine.

The trend occurred in the expected direction, with an increase in HTL associated with a reduction in TEOAE level, although there was wide variation. The highest correlations occurred between TEOAE frequencies at 2 or 3 kHz and HTL at 1 kHz. The high correlations at a TEOAE frequency of 2 kHz are consistent with other studies of TEOAE level and HTL (Gorga et al, 1993b). Previous cross-sectional studies have attributed the high correlation of HTL with TEOAE at 2 kHz to the concentration of TEOAE energy at this frequency due to middle ear factors. However in this study when the middle ear factors were controlled for using a longitudinal design, changes in HTL were still related to changes in TEOAE at 2 kHz. This may be because TEOAE energy is greatest around this frequency and so the largest changes were detected at 2 kHz.

This frequency relationship was consistent with studies of OAE and noise exposure where the OAE frequency correlated to HTL was approximately 1 octave higher than the frequency of the hearing damage (Engdahl and Kemp, 1996). The group correlation between the TEOAE and HTL variables was higher using TEOAE evoked by the lower intensity stimulus levels. This was consistent with other studies showing the lower stimulus levels more sensitive than higher levels (Marshall and Heller, 1996).

The relationship between the longitudinal changes in TEOAE and HTL was similar to the cross-sectional study results, and did not show the expected improvement as a result of the longitudinal experimental design. Therefore the hypothesis was not upheld. However in a similar way to the DPOAE results, individual subjects and ears showed highly significant relationships between TEOAE level and HTL. This showed a direct relationship between changes in TEOAE and HTL in

approximately 50% of ears, for a wide range of frequency combinations. For those ears that showed significant relationships between TEOAE level and HTL, the changes in TEOAE level mostly occurred concurrently with changes in HTL and the change in TEOAE mostly did not precede the change in HTL. However the changes were not always frequency specific to HTL.

There was wide variation in the combination of variables that were significantly related. Some ears showed significant relationships for most combinations of variables, whereas others showed significant relationships for only a few variables. The most common combination of variables was between the broadband and 1 kHz TEOAE responses and HTL at 3 kHz. Although this pattern of results was generally observed, there was wide variability among ears and there were significant correlations between most TEOAE and HTL frequency combinations.

Fifty percent of ears did not show a significant relationship between TEOAE and HTL and to explain the results of the whole group other factors that are not common to TEOAE and HTL are likely to be involved in the relationship. The framework, which was based on Mills' model for DPOAE I/O functions, does not appear to be appropriate for TEOAE. This may be due to the different generation mechanisms of TEOAE compared to DPOAE (Shera and Guinan, 1999). TEOAE generation requires cochlear amplification and reflection sites. Distortion may also be required (Yates and Withnell, 1999; Talmadge et al, 2000). It is possible that there were changes in the reflection sites or nonlinearities, unrelated to a change in cochlear amplification that led to change in TEOAE without a related change in HTL.

Alternatively, pre-audiometric changes in TEOAE may be measuring OHC damage at higher frequency places along the BM that are responsible for TEOAE generation (i.e. at places along the BM away from the characteristic place of the audiometric frequencies). Desai et al. (1999) showed that subjects with normal hearing but significant exposure to noise had reduced TEOAE or absent TEOAE, compared to subjects with normal hearing and no significant noise exposure. Subjects in the noise exposed group are likely to have high frequency hearing loss, possibly at frequencies above those included within the TEOAE spectrum. Similarly Attias and Bresloff (1996) measured changes in TEOAE level but no changes in HTL. It is possible that rather than measuring frequency specific changes in OHC function that have not yet affect the HTL, that TEOAE are measuring changes in cochlear function at areas along the BM not tested by PTA, particularly high-frequency areas. This is particularly likely for studies of TEOAE in subjects undergoing ototoxic drug treatment. Chemotherapy drugs particularly are known to affect the extra high frequency hearing thresholds, and changes in CA gain at these frequencies are likely to affect lower frequency TEOAE generation.

The lack of frequency specificity between changes in TEOAE and HTL is contrary to the predicted framework, and implies that changes in hearing at a characteristic frequency affect TEOAE level at other frequencies or that TEOAE at a particular frequency are correlated with changes at other

frequencies. One explanation is that distortion as well as reflection has a role in TEOAE generation, as proposed by Yates and Withnell (1999). This is also in agreement with the results of Avan et al (1997), who showed that HTL frequencies greater than the frequencies within the TEOAE spectrum accounted for the variation in TEOAE frequency response. New methods of recording TEOAE using an open ear technique have been shown to improve the recording of the TEOAE response at the higher frequencies (Withnell et al, 1998; Merritt and Kapadia, 2003). It would be interesting to repeat this experiment using this technique to measure more of the higher frequency components of the TEOAE spectrum. It is possible that higher frequency TEOAE will be correlated with HTL at even higher frequencies than currently measured.

HTL changes were also well correlated with changes in the BB TEOAE response. The broadband TEOAE response is likely to consist of contributions from the BM at least between 1 and 4 kHz, and also at more basal frequencies (Avan et al, 1997). Changes in CA function at any place along the BM are therefore most likely to be reflected in the TEOAE measure that includes this area in its generation. This may account for the fact that the BB responses showed the most significant relationships with different HTL frequencies. It may also explain why it was not possible to predict which subjects would show the best relationship with HTL. Although the effect of salicylate on hearing was only measured at audiometric frequencies, it is likely to have had an effect on hearing at other characteristic frequencies along the BM that were not measured.

It was not possible to identify any factors that predicted which subjects and which TEOAE/HTL variables would show significant relationships. There was no link to the size of the change of TEOAE level or HTL, or the initial TEOAE or HTL values. As was shown for the DPOAE results, for particular combinations of variables, some subjects showed significant correlations whereas other subjects showed no significant relationship. This ensured that when the group data were analysed the overall correlation was low. Many previous studies that have looked at the change in TEOAE level versus the change in HTL have taken only two measures: pre- and post- exposure measures (e.g. Engdahl et al, 1996). Predictably, they showed no strongly significant group relationship. The results of the group analysis of this experiment (measuring the change in HTL versus the change in TEOAE) were similar if not slightly better than those recorded in other TTS experiments: Sliwinska-Kowalska et al (1999) recorded the change in TEOAE level in factory workers exposed to noise levels as high as 97 dB(A) for 6 hours. They reported no significant relationship between the size of the noise-induced TTS and the change in TEOAE level. However they used the ILO288 in the nonlinear mode and 80 dB SPL clicks. The improved correlation values measured in this experiment were obtained using lower intensity click levels.

Analysis of the rate of change of TEOAE and HTL within ears with significant relationships showed wide variability in the rate of change of TEOAE with HTL. Unlike DPOAE in which the rate of change was similar between ears (with significant relationships) the variability was higher for the

TEOAE data. The slope of the relationship between the TEOAE and HTL for these ears was more variable than for DPOAE. This implies that the effect of a change in HTL on TEOAE is variable between ears. This may be related to the complex nature of TEOAE generation and the likely non-frequency-specific nature of the TEOAE response. A change in CA function at any place along the BM may have an effect at varying places along the spectrum of the TEOAE and therefore the relationship between a particular CA frequency and level of the TEOAE is more difficult to detect. For this reason TEOAE may be very useful for detecting any change along the BM, but not for identifying the location.

Therefore, based on the results of the whole group, the hypothesis that longitudinal changes in TEOAE and HTL show a higher correlation than cross-sectional differences must be rejected. Although in some subjects this hypothesis was upheld, the variability between subjects in the relationship between TEOAE and HTL necessitate that it must be rejected. These results show that for some subjects, a simple model in which TEOAE level is directly related to hearing threshold is sufficient. However to encompass all subjects a more complex model is required. Based on these results the framework proposed for TEOAE based on Mills' model of I/O functions appears to be unsuitable.

6.7.4 MLS TEOAE

The effect of salicylate on MLS TEOAE was similar to the results obtained for the conventional TEOAE. For this reason, the discussion of MLS TEOAE is limited to differences between the two recording techniques, as much that applies to MLS TEOAE was discussed in the previous discussion of conventional TEOAE.

Salicylate had a significant effect on MLS TEOAE level. In general, the largest changes in level occurred at the lower click stimulus intensity levels of 50 and 60 dB, although there were reductions across the click intensity range. MLS TEOAE had the advantage over the conventional TEOAE recording method in that responses were recorded down to the lower click level of 50 dB, which showed the largest level changes. The largest changes in level were measured at 2 and 3 kHz. Both MLS and conventional TEOAE showed similar magnitude of level changes.

MLS TEOAE level evoked by both high and low click intensity levels were sensitive to salicylate. There was no mean change in nonlinearity at 1 kHz, and only a small mean decrease in nonlinearity at the higher frequencies and the broadband response. At 3 kHz, and to a lesser extent at 2 kHz, the I/O function showed an overall downward shift with salicylate. There were smaller changes in the slope values of the I/O function than measured for conventional TEOAE.

Following the model of TEOAE I/O functions, the change in MLS TEOAE was estimated using two different methods: measuring the change in MLS TEOAE level at a constant stimulus level and

measuring the change in stimulus intensity at a constant MLS TEOAE level. The relationship between MLS TEOAE and HTL was evaluated.

It was hypothesised that changes in MLS TEOAE would have a higher correlation with HTL than changes in conventionally recorded TEOAE. Analysis of the group changes in MLS TEOAE level showed several significant correlations with changes in HTL. The results were similar to the conventional TEOAE where the highest correlations were with HTL at 1 and 2 kHz and TEOAE at 2 and 3 kHz. The stimulus level at each frequency that showed the highest correlation was 60 dB. Although there were several significant correlations, in most cases the variance explained was only 30% or less. These correlations were no higher than the conventional TEOAE results. Longitudinal changes in MLS TEOAE and HTL were also expected to have a higher correlation than cross-sectional differences. However comparison of the results showed similar correlation coefficients for the two experiments.

Individual subjects and ears showed significant relationships between MLS TEOAE level and HTL. This showed a direct relationship between changes in MLS TEOAE and HTL in approximately 50% of ears, for a wide range of frequency combinations. There was wide variation in the variables that were significantly correlated. The most common combination of variables was MLS TEOAE at 2 kHz and HTL at 3 kHz.

It was difficult to calculate the changes in *MLS TEOAE stimulus level* for most ears. The downward shifts in I/O functions meant that it was not possible to calculate the change in stimulus level at MLS TEOAE frequencies of 2 kHz and above. Analysis of the broadband and 1 kHz responses showed no significant group effect. There were significant individual effects, as for the conventional TEOAE results.

The rate of change of MLS TEOAE with HTL showed high variability between ears. There was no particular effect of level on the rate.

6.7.5 MLS TEOAE rate suppression

There were changes in MLS TEOAE rate suppression with salicylate. Rate suppression was generally smallest at a click level of 80 dB, but results at this click level showed the largest changes with salicylate consumption. Rate suppression at 1 kHz showed the largest changes, caused by a smaller reduction in level at 5000 clicks/s compared to 50 clicks/s. This was not observed at 3 kHz, where the change in level was equal at both click rates. The maximum change in rate suppression was 10 dB.

The framework predicts a reduction in rate suppression with salicylate, due to an overall reduction in MLS TEOAE level, greater at the lower click rate than the higher click rate. This was upheld up to 2 kHz. At 3 kHz the results were not consistent with the framework as there was an equal reduction in MLS TEOAE at the low and high click rate, resulting in no change in rate suppression with salicylate.

This result is consistent with the TEOAE I/O functions at 3 kHz, which showed a consistent change in level across the stimulus range.

It was difficult to assess the relationship between the change in I/O function slope and rate suppression because there was little change in MLS TEOAE I/O function nonlinearity with salicylate. The greatest changes in nonlinearity were measured for I/O functions at 2 and 3 kHz. Correlation of the change in I/O function slope with the change in rate suppression showed mildly significant results at some frequencies. The results were mostly frequency specific, with a reduction in rate suppression associated with an increase in I/O function slope at the same frequency. In general a 0.1 dB/dB increase in function slope was associated with a 1 dB decrease in rate suppression. The highest correlations were obtained for the broadband responses, but the maximum correlation coefficient was only –0.63. Interestingly, when all data across the seven sessions were combined, the highest correlation was found for the broadband responses and at 2 and 3 kHz. These correlation coefficient values were higher than those obtained relating the changes in I/O function slope and rate suppression. These results are consistent with those obtained for the cross-sectional study.

The relationship between the change in MLS rate suppression and HTL was investigated. It was expected that the longitudinal changes in rate suppression would show a higher correlation with HTL than the cross-sectional differences. The group relationship of the change in rate suppression versus the change in HTL gave similar correlation coefficients to other OAE measures. The highest correlation was obtained between the broadband rate suppression and HTL at frequencies of 3 and 4 kHz. However the maximum coefficient value was –0.56. This was similar to the cross-sectional differences measures in Experiment.

As was observed for other OAE measures, there were several significant relationships between rate suppression and HTL for individual subjects and ears. This showed a direct relationship between changes in MLS rate suppression and HTL in approximately 30% of ears, for a wide range of frequency combinations. This was a lower number of correlations than obtained with the other OAE measures, and particularly MLS TEOAE. Therefore, as for the other OAE measures, other factors are important in the relationship between MLS rate suppression and HTL.

This provides further evidence that MLS TEOAE rate suppression is of cochlear origin. Nonetheless MLS rate suppression and MLS TEOAE level were not always related to the same HTL frequencies, indicating that these two measures may be measuring different cochlear effects.

6.7.6 SOAE

30% of subjects, and 24% of ears had measurable SOAE. 29% of the SOAE were recorded in the left ear and 71% in the right ear. The prevalence values are lower than other studies of SOAE prevalence, but the higher number of SOAE in the right ear is consistent with other studies (Penner et al, 1997).

These lower prevalence values are likely to be a result of the equipment used, which was not designed specifically to detect SOAE and may have underestimated the number of SOAE.

Subjects with SOAE had on average larger DPOAE, TEOAE and MLS TEOAE than subjects with no detectable SOAE. These differences were larger at the lower frequencies, closer in frequency to the SOAE. This is consistent with other studies (Kulawiec and Orlando, 1995; Osterhammel et al, 1996; Ozturan and Oysu, 1999).

The changes in SOAE with aspirin were consistent with the study by McFadden and Plattsmier (1984). Of the eight ears with recordable SOAE, seven SOAE were abolished with salicylate. The SOAE of the remaining ear was still recordable above the noise floor during salicylate ingestion.

There was no difference in the effect of salicylate on OAE in subjects with and without SOAE. There was also no difference between subjects with and without SOAE in the number of significant correlations between HTL and different OAE measures. SOAE in this study were therefore not a significant factor influencing the relationship of HTL and OAE.

6.7.7 Comparison of DPOAE and TEOAE

Shera and Guinan (1999) predicted that salicylate would have a greater effect on TEOAE than DPOAE. They theorised that salicylate reduces the reflection processes involved in TEOAE generation but does not affect the nonlinear force generation of the OHC, thought to have the greatest involvement in the generation of DPOAE; hence there should be a greater change in TEOAE rather than DPOAE level. Berninger and Gustafsson (2000) in their study of the effects of quinine on TE/DPOAE, albeit using a limited set of stimulus parameters, upheld the prediction of Shera and Guinan in which they found a close correspondence between TEOAE and HTL but not DPOAE and HTL.

However this study showed similarities in the mean change in level of the two OAE types with salicylate, and this prediction was not upheld. At most stimulus levels DPOAE and TEOAE showed similar changes in level. However the largest changes in level overall occurred for DPOAE evoked by L2 levels of 40 dB, and this was greater than any of the TEOAE changes. Salicylate therefore had the greatest effect on low intensity stimuli DPOAE, but the effects were similar for the mid-intensity stimulus DP and TEOAE. It is possible that if TEOAE had been measured at even lower intensity stimuli, the change at this lower level would have been similar to that of the DPOAE. An 80 dB TEOAE stimulus stimulates the whole cochlea, and the stimulus level at a particular frequency band will be less. Therefore it is not meaningful to directly compare the same stimulus levels for DP and TEOAE.

It is argued that salicylate has a lesser effect on DPOAE because, although the amplification of the TW may be reduced, the nonlinearities that are required for distortion generation are unlikely to be

affected by salicylate. By this argument, salicylate will have a greater effect on the reflection component of DPOAE generation than on the distortion part. However for the stimulus parameters used in this experiment ($f_2/f_1 = 1.2$), the reflection component of DPOAE level is probably small compared to the distortion component (Knight and Kemp, 1999).

The fact that salicylate had a similar effect on DPOAE as TEOAE could be interpreted in two ways: it may indicate that salicylate has a greater effect on the distortion component of DPOAE generation, similar to the effect on the reflection component affecting TEOAE generation. Alternatively it could be that TEOAE has a greater distortion component to its generation mechanism, and that the similarities in the DP and TEOAE reduction are a result of similar distortion generation components that are affected equally by salicylate. Salicylate has been shown experimentally in animals to affect the electromotility of OHC and also basilar membrane mechanics (Karlsson and Flock, 1990; Cazals, 2000), and it is possible that it has an effect on the OHC force generation and hence distortion nonlinearities.

These results with salicylate are different to those obtained by Berninger et al (1995), who compared the differential effect of quinine on TEOAE and DPOAE level. Their experiment examined only a small number of variables and both types of OAE were evoked by high stimulus levels only. DPOAE were evoked with L1 and L2 equal to 75 dB SPL, and showed a mean reduction of approximately 2 dB with quinine. TEOAE were evoked by a click level of 79 dB SPL, and were reduced by approximately 5 dB. The changes in DPOAE level recorded by Berninger et al (1995) are similar to the results of this experiment, but their TEOAE results were reduced by 3-4 dB more than those recorded here. As they did not evoke OAE at lower intensity levels it is not possible to make any other comparisons. A later study by Berninger and Gustafsson (2000) examined the effect of quinine on DPOAE evoked across a range of stimulus levels. They showed a mean change in DPOAE level of 1 dB at 70 dB SPL stimulus level increasing to 10.5 dB at 40 dB SPL stimulus level. The changes at the lower intensity stimulus levels in DPOAE level with quinine are larger than those recorded in this study with salicylate. This may be a result of differences in the physiological effect of quinine and salicylate on the cochlea. They recorded pure tone threshold changes up to 30 dB, so it is likely that quinine (using the doses specified) had a stronger effect on the cochlea than the effect of salicylate in this experiment. Unfortunately they did not record TEOAE concurrently.

The relationship between change in DP and TEOAE were examined. Correlation analysis of changes DP and TEOAE level showed only a weak correlation between changes in the two OAE types with aspirin consumption. The correlations were highest at low-to-moderate DPOAE stimulus level and TEOAE evoked by stimuli levels of 60-80 dB SPL. This implies that salicylate is having a differential effect on the two types of OAE within subjects.

The results of this experiment were compared to the results obtained by Knight and Kemp (1999), who examined the relationship between TEOAE, $2f_1-f_2$ and $2f_2-f_1$ DPOAE level. They used one TEOAE stimulus level (0.3 Pa = approx 80 dB SPL) and recorded TEOAE using the nonlinear subtraction method. Their results were expressed in terms of the standard deviation (SD) of the residuals in the linear regression calculation, rather than the correlation coefficient. Knight and Kemp (1999) showed the highest correlation between DP and TEOAE at the lower DPOAE stimulus levels, as shown here. Overall, their results showed the highest correlation between TEOAE and $2f_2-f_1$, and also between TEOAE and $2f_1-f_2$ evoked using a low f_2/f_1 frequency ratio. TEOAE and the $2f_1-f_2$ distortion product at an f_2/f_1 ratio of 1.2 showed a low correlation.

Comparison of the pre- and peri- salicylate results showed that salicylate was able to improve the relationship between DP and TEOAE. This only occurred when the TEOAE frequency was lower than the DPOAE frequency. The slope of the regression line relating DPOAE and TEOAE approached 1 dB/dB during salicylate exposure. Salicylate appears to be having an effect to cause TEOAE to be more like DPOAE. The improved correlation between the two OAE types implies that their underlying mechanisms are more similar after salicylate ototoxicity; that salicylate is altering a particular mechanism of OAE generation to make the two OAE types more similar. This may be a change in the reflection process of TEOAE generation, leaving only the intermodulation distortion component. However if this were the case, then the increase in correlation between DP and TEOAE would be expected to occur at all frequencies. The fact that only TEOAE at lower frequencies than DPOAE are more alike implies that the similarities may be mediated through intermodulation distortion, propelled in an apical direction. A reduction in cochlear function at a high frequency place along the BM could have an impact on TEOAE production via a change in the intermodulation distortion, at a lower frequency. It is unlikely that an apical change in BM function could affect the basal TW, whereas it is possible that a basal change could affect the apex. It may also be an increase in the reflection compared to the distortion generation mechanism of DPOAE with salicylate. Knight and Kemp (1999) showed that the $2f_2-f_1$ DPOAE is more closely related to TEOAE than the $2f_1-f_2$ DPOAE. They postulate that this is due to the greater reflection component of the $2f_2-f_1$ DPOAE, making it more like TEOAE than the $2f_1-f_2$ DPOAE. Salicylate may therefore be increasing the proportion of reflection to distortion mechanisms in DPOAE generation. It would be helpful to examine phase changes to understand these changes in OAE generation.

Examination of the changes in DP and TEOAE showed that in most cases the changes were concurrent. At 2 and 3 kHz most subjects showed changes in both of the OAE types. It was rare to find subjects with changes in one OAE type without a change in the other OAE. This indicates that both OAE types were sensitive to salicylate. However the fact that there was only a low correlation between changes in DP and TEOAE indicates that the salicylate is having a different effect on both types of OAE generation mechanisms.

In terms of the relationship with HTL, there were no similarities between DPOAE, TEOAE and their relationship with HTL frequencies. From the prediction of Shera and Guinan (1999) it was expected that TEOAE would have a higher correlation with a change in HTL than DPOAE. However this was not observed. Both DPOAE and TEOAE showed significant relationships with HTL. Within individual ears the two OAE types showed different relationships with HTL, implying they were measuring different changes in cochlear function.

For TEOAE, the broadband response appears to be related to HTL as well as any of the frequency banded TEOAE. This implies that TEOAE is not particularly frequency-place specific.

Cochlear amplification for TEOAE generation is responsible for the tall and broad TW, which filters the reflected wavelets off the perturbations and generates the backward TW, which is amplified as it traverses the BM and out of the ear. Salicylate is hypothesised to have several effects at points along this generation process. It has an effect on the OHC responsible for the dense perturbations that probably act as the reflection site. It may thus reduce the potential reflection sites. Secondly, by acting on these perturbations responsible for the cochlear amplification that generates a finely tuned TW, it may reduce the gain of the TW, thus reducing the tall/broad shape responsible for giving coherence to all the wavelets. Thirdly, it may act on the cochlear amplification process on the backward TW thus reducing its level. If distortion is also involved, it will also reduce the intermodulation interaction. The mechanism of salicylate action is potentially complex, and may affect OAE generation at a number of sites.

The more consistent rate of change of DPOAE with changing HTL implies that DPOAE generation has the closest relationship to auditory sensitivity. TEOAE may be more sensitive to changes in hearing at more places along the BM than DPOAE, and are therefore more sensitive and less frequency specific, giving inconsistent rates of change across subjects. The wave-fixed nature of DPOAE may mean they are less sensitive to changes in CA gain at other places along the BM, and therefore are more specific to changes in HTL at particular frequencies. This could account for the greater variability in TEOAE versus HTL, than DPOAE versus HTL.

The question whether wave fixed or place fixed OAE give the best measure of auditory status has been discussed in the literature. However both DP and TEOAE are thought to be a combination of both generation mechanisms (Shera and Guinan, 1999; Yates and Withnell, 1999; Knight and Kemp, 1999). There are similarities between the two types of OAE in terms of level, but there are marked differences in their relationship with HTL.

6.8 CONCLUSIONS

The thesis is based on the assumption that there is close relationship between OAE and HTL mediated through the cochlear amplifier. Leading on from this, it was hypothesised that one of the reasons that moderate correlations were measured in previous studies (e.g. Gorga et al, 1993a,b) were a result of inter-subject and inter-ear variables, such as middle ear factors influencing the relationship. This longitudinal study aimed to test the hypothesis that changes in OAE and HTL would show a higher correlation than differences between OAE and HTL.

The results of the longitudinal study showed that overall, the strength of the relationship between changes in OAE and HTL was similar to the cross-sectional study, and the hypothesis that longitudinal changes have a higher correlation than differences in OAE and HTL was not supported. This was true for the DPOAE, TEOAE and MLS TEOAE measures, and also for MLS TEOAE rate suppression measures.

However for approximately 50% of ears, there were highly significant relationships between the changes in OAE and HTL that was higher in correlation than the cross-sectional results. This was found for DP, TEOAE and MLS TEOAE rate suppression. There was a wide range of frequency combinations between the OAE and HTL measures. For these ears the hypothesis holds, and suggests that inter-ear differences are responsible for some of the variation in OAE for some ears. However as this was not applicable to all subjects it seems likely that the main assumption of the thesis is false, and that other factors are important in the relationship between OAE and HTL. Other factors are therefore required for the model relating OAE and HTL, which may include inner hair cells and other cochlear factors and work is required to differentiate the relative importance of these.

The relationship between changes in DP and TEOAE were compared and the prediction of Shera and Guinan (1999) tested that TEOAE are more sensitive to salicylate. This hypothesis was not upheld in this experiment as both OAE types showed similar changes in level with salicylate.

This experiment suggests that some of the variability in the cross-sectional studies of OAE and HTL are compounded by inter-ear differences that affect OAE and HTL differently. These may include anatomical and physical differences in the external and middle ear of subjects. However other factors are also involved, possibly cochlear factors, and also intrinsic subject factors, which are not controlled for in longitudinal studies. More research is required to develop a more complex model that encompasses all subjects and ears and may including these other factors.

7 COMPARISON OF THE CROSS-SECTIONAL AND LONGITUDINAL STUDIES

7.1 INTRODUCTION

The results of the longitudinal study were compared to the results of the cross-sectional study. This was to assess whether differences in OAE/HTL between subjects were similar to changes in OAE/HTL within subjects, and to answer the question as to whether results from cross-sectional studies can be used to infer changes within subjects. It is acknowledged that the small hearing shifts (<20 dB) obtained in the longitudinal study makes it difficult for a direct comparison with the cross-sectional study, which contains a wider spread of HTL values.

The variable chosen for comparison was the slope of the relationship between OAE and HTL. It was hypothesised that these would be similar for the two different studies. The comparison took two forms: firstly comparing the cross-sectional data to the full group longitudinal data (all subjects included), and secondly by comparing the cross-sectional data to the selected longitudinal data (only subjects with significant relationships between OAE/HTL included).

7.2 DPOAE

The relationship between DPOAE level and HTL measured in the cross-sectional study was compared to the results obtained in the longitudinal study. The slope values of the linear regression analyses relating DPOAE level (independent variable) and HTL (dependent variable) from the two experiments are summarised in Table 7-1.

The comparison was made in two parts. Firstly a comparison was made of the cross-sectional results with the full group longitudinal data: this showed little similarity in the relationship between DPOAE and HTL for the two types of study. Whereas the cross-sectional study showed many significant correlations, there were few significant correlations measured in the group longitudinal data. At the frequencies where there where significant relationships for both the cross-sectional and longitudinal studies, the slope of the relationship between DPOAE and HTL was markedly different. Figure 7-1 compares graphically the cross-sectional and group longitudinal relationships of DPOAE measured at 3 kHz (L2=50 dB) and HTL at 3 kHz. There was wide variation within the data from the longitudinal study. The shifts due to aspirin were small, and so it is difficult to compare trends. The slope of the longitudinal regression line was approximately half that measured in the cross-sectional study. The regression line plotted from the cross-sectional data is influenced strongly by subjects with mild/moderate hearing impairment. These are greater than the temporary hearing losses induced in the longitudinal study.

Table 7-1: Comparison of the slope values from linear regression analysis relating DPOAE level (independent variable) and HTL (dependent variable) in the cross-sectional and longitudinal studies

OAE (frequency/ L2 level)	HTL frequency (kHz)	Slope value of linear regression analysis (dB/dB)		
		Cross-sectional	Longitudinal (full group)	Longitudinal (median value: selected subjects)
3 kHz/40 dB	3	–0.91	NS	–1.00
3 kHz/50 dB	3	–0.82	–0.35	–1.34
3 kHz/60 dB	3	–0.78	NS	–2.87
4 kHz/40 dB	4	–1.02	NS	–1.43
4 kHz/50 dB	4	–0.92	NS	–2.45
4 kHz/60 dB	4	–1.12	NS	–2.27
6 kHz/40 dB	6	–1.26	NS	–0.73
6 kHz/50 dB	6	–0.98	NS	–2.49
6 kHz/60 dB	6	–0.70	NS	–4.43

Key to abbreviations – NS: not significant.

Secondly comparing the cross-sectional results with the selected longitudinal data: it was predicted that the slopes relating the OAE and HTL would be similar between the two studies. This showed differing slope values in the relationship between DPOAE and HTL for the two types of study. The cross-sectional slope values did not vary considerably with DPOAE stimulus level, whereas the longitudinal data showed a progressive increase in slope value with increasing DPOAE stimulus level. The slope values were most similar between the two types of study at the lowest DPOAE stimulus levels. Figure 7-2 compares the cross-sectional data to the changes in DPOAE/HTL for individual subjects. In this example, for subjects 9 and 10, the change due to aspirin is similar to the cross-sectional differences. However for subject 14 the longitudinal change is quite different to the cross-sectional data, as this subject shows a small decrease in DPOAE level corresponding to a large change in HTL.

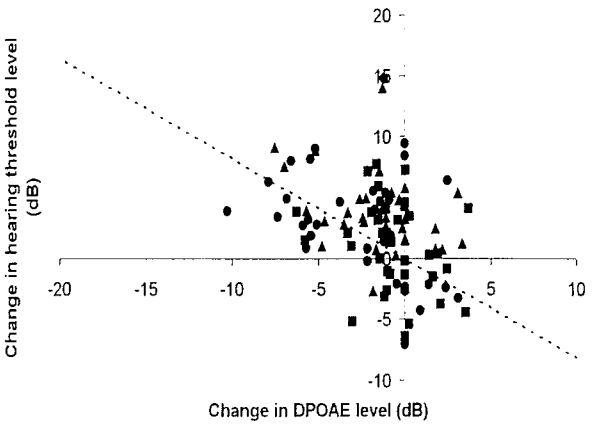


Figure 7-1: Longitudinal changes in DPOAE level (3 kHz response to 60/50 primaries) and 3 kHz HTL. All longitudinal subject data included. These data are compared to the cross-sectional data (regression line plotted of cross-sectional differences in DPOAE level against HTL). Key to symbols – squares show longitudinal changes at session 3, triangles at session 4 and circles at session 5.

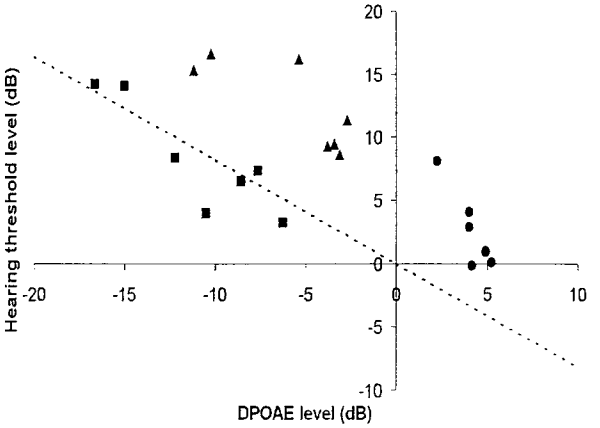


Figure 7-2: Comparison of individual subject longitudinal data with the cross-sectional data (regression line). DPOAE level measured at 3 kHz to 60/50 primaries versus HTL 3 kHz. Dashed line represents the regression line from the cross-sectional study. Key to symbols – from the longitudinal study, squares: subject 9, triangles: subject 10, circles: subject 14.

7.3 TEOAE

The relationship between TEOAE level and HTL measured in the cross-sectional study was compared to the results obtained in the longitudinal study. The results are summarised in Table 7-2.

As described for DPOAE, comparison of the cross-sectional data with the group longitudinal data showed little similarity between the two studies. There were only a few significant relationships between TEOAE and HTL in the group longitudinal study but many significant relationships in the cross-sectional study. The lack of significant relationships in the group longitudinal is likely to be due to the small shifts in HTL. Figure 7-3 compares graphically the cross-sectional and group longitudinal relationships of broadband TEOAE level (60 dB click level) and HTL at 3 kHz. There was wide variation within the longitudinal data, and due to the small changes in TEOAE level from aspirin it is difficult to estimate whether they are consistent with the cross-sectional data.

Secondly cross-sectional data were compared to the selected longitudinal study data. The median slope values from the selected longitudinal study were generally higher than the slope values from the cross-sectional study. As described for DPOAE, the values were most similar at the lower DPOAE stimulus levels. Figure 7-3 compares the cross-sectional data to the changes in TEOAE/HTL for

individual subjects. All three subjects in the example show changes due to aspirin that are similar the cross-sectional differences.

Table 7-2: Comparison of the slope values from linear regression analysis relating TEOAE level (independent variable) and HTL (dependent variable) in the cross-sectional and longitudinal studies

OAE (frequency/click level)	HTL frequency (kHz)	Slope value of linear regression analysis (dB/dB)		
		Cross-sectional	Longitudinal (full group)	Longitudinal (median value of selected subjects)
BB/60 dB	3	–1.03	NS	–1.99
BB/70 dB	3	–1.23	NS	–3.24
BB/80 dB	3	–1.04	NS	–2.24
1 kHz/60 dB	3	–1.42	NS	–1.64
1 kHz/70 dB	3	–1.35	NS	–3.20
1 kHz/80 dB	3	NS	NS	–0.87
2 kHz/60 dB	3	NS	NS	–1.28
2 kHz/70 dB	3	–1.14	–1.32	–2.35
2 kHz/80 dB	3	–1.23	NS	–3.02
2 kHz/60 dB	4	NS	–2.08	NS
2 kHz/70 dB	4	–1.34	NS	–1.97
2 kHz/80 dB	4	–1.55	NS	–3.86
3 kHz/60 dB	4	NS	NS	–1.51
3 kHz/70 dB	4	–1.32	NS	–1.73
3 kHz/80 dB	4	–1.34	NS	–2.11

Key to abbreviations – BB: broadband, NS: not significant.

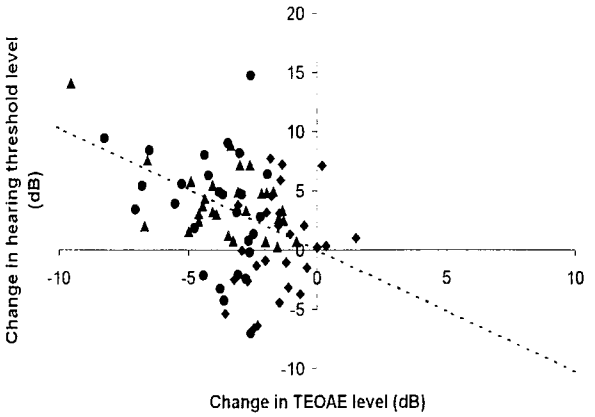


Figure 7-3: Longitudinal changes in TEOAE level (broadband response,60 dB click stimulus) and 3 kHz HTL. All longitudinal subject data included. These data are compared to the cross-sectional data (regression line plotted of cross-sectional differences in TEOAE level against HTL). Key to symbols - squares show longitudinal changes at session 3, triangles at session 4 and circles at session 5.

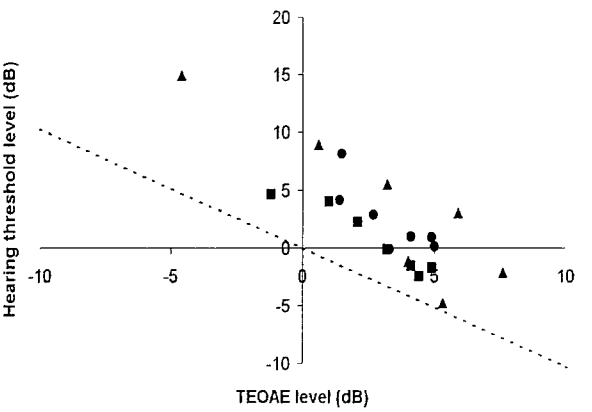


Figure 7-4: Comparison of individual subject longitudinal data with the cross-sectional data (regression line). TEOAE broadband response (60 dB) versus HTL 3 kHz Dashed line represents the regression line from the cross-sectional study. Key to symbols - from the longitudinal study, squares: subject 8, triangles: subject 13, circles: subject 14.

7.4 MLS TEOAE

The relationship between MLS TEOAE level and HTL measured in the cross-sectional study was compared to the results obtained in the longitudinal study. The results are summarised in Table 7-3.

The results were similar to the conventionally recorded TEOAE results. There was no similarity between the cross-sectional data and the group longitudinal data. Again, this may be related to the small shifts in HTL recorded in the longitudinal study. Figure 7-5 compares graphically the cross-sectional and group longitudinal relationships of MLS TEOAE recorded at 3 kHz (80 dB click level) and HTL at 4 kHz. There was wide variation within the longitudinal data, and due to the small shifts it was difficult to compare with the cross-sectional data.

The cross-sectional data was compared to the selected longitudinal data. The median slope values of the selected longitudinal study were generally higher than the slope values from the cross-sectional study. Figure 7-6 compares the cross-sectional data to the changes in MLS TEOAE/HTL for individual subjects. These individual subject examples showed changes in MLS TEOAE consistent with the cross-sectional differences.

Table 7-3: Comparison of the relationship between MLS TEOAE level (5000 clicks/s) and HTL in the cross-sectional and longitudinal (group) experiments

OAE (frequency/click level)	HTL frequency (kHz)	Slope value of linear regression analysis (dB/dB)		
		Cross-sectional	Longitudinal (all group)	Longitudinal (selected subjects)
BB/50 dB	3	–1.40	NS	–2.00
BB/60 dB	3	–1.26	NS	–2.20
BB/70 dB	3	–0.97	NS	–1.82
3 kHz/50 dB	3	–0.92	NS	–1.23
3 kHz/60 dB	3	–0.79	NS	–1.39
3 kHz/70 dB	3	–0.94	NS	–1.90
4 kHz/50 dB	4	–1.46	NS	–2.04
4 kHz/60 dB	4	–1.63	NS	–1.54
4 kHz/70 dB	4	–0.94	NS	–1.62

Key to abbreviations – BB: broadband, NS: not significant.

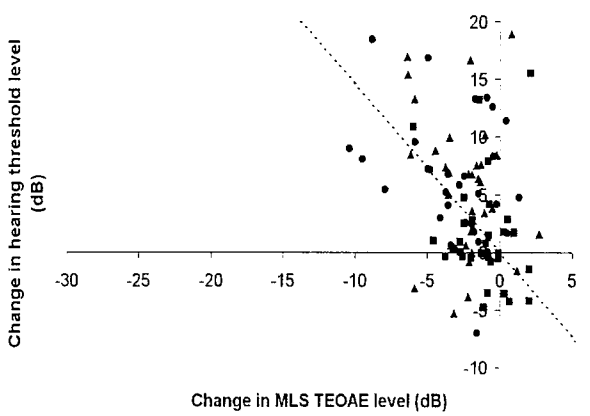


Figure 7-5: Comparison of cross-sectional data (regression line) and longitudinal data from the MLS TEOAE at HTL of 4 kHz (3 kHz response to a 80 dB click stimulus). Key to symbols – squares: changes at session 3, triangles: session 4, circles: session 5.

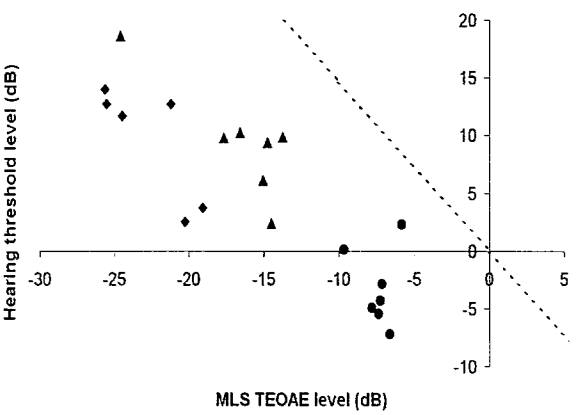


Figure 7-6: MLS TEOAE (3 kHz, 50 dB) versus HTL (3 kHz) Dashed line represents the regression line from cross-sectional study Key: From longitudinal study squares subject 3, triangles subject 13, circles subject 7.

7.5 DISCUSSION

The results of both studies showed wide variation in the relationship between OAE and HTL.

Individual subject DP, MLS TEOAE and TEOAE data from the longitudinal and the cross-sectional study all showed some support for the expected association between changes in OAE level and HTL. However, when grouped together there were wide variations across OAE type and frequency, suggesting a simple quantitative model of the association is inadequate. Moreover, comparison between the two studies was hampered by the small magnitude of aspirin-induced shifts in the longitudinal study, compared to the cross-sectional study differences.

It was hypothesised that longitudinal changes in HTL would have a higher correlation with changes in OAE than cross-sectional differences in HTL and OAE. The relationship between HTL and the corresponding OAE level was compared between the two studies. The comparison was held back by the fact that there were no subjects with HTL greater than 20 dB in the longitudinal study. HTL and OAE measures showed a broadly similar trend of decreasing OAE level with increasing HTL, in both the cross-sectional and longitudinal studies. However, the small shifts observed in the longitudinal study and the variation amongst subjects meant that the trends observed must be treated with some caution.

The group analysis of the longitudinal experiment gave similar results to those of the cross-sectional study. This shows that even when the aetiology of the hearing loss is controlled for (salicylate induced in experiment 2), there is still wide inter-subject variability.

Nonetheless, the study suggests that differences between subjects broadly reflect changes that occur within subjects in terms of OAE level and HTL. Changes that occurred within some individual subjects showed high correspondence to the cross-sectional differences.

Cross-sectional studies of differences between subjects therefore have importance to infer changes within subjects that cannot be obtained experimentally in humans for ethical reasons. In the longitudinal study, there were several examples of salicylate-induced changes in TEOAE with no corresponding change in HTL. This suggests that TEOAE may be sensitive to sub-audiometric changes in cochlear function, possibly unrelated to cochlear amplification.

8 SUMMARY AND CONCLUSIONS

The overall aim of the thesis was to explore in detail the relationship between otoacoustic emissions (OAE) and hearing threshold level (HTL) in human subjects. The main assumption of the thesis was that there is a close relationship between OAE and HTL based on the assumption that OAE require outer hair cells (OHC) and cochlear amplification for generation (Talmadge et al, 1998; 2000; Kemp, 2002) and HTL, up to and including mild sensorineural hearing loss, is predominantly due to OHC loss (Patuzzi et al, 1989). Therefore a high correlation was expected in the relationship between OAE and HTL.

The alternative to this assumption was that other factors are important for both OAE generation and HTL. For OAE generation, these other factors may include cochlear nonlinearities and inhomogeneities, and for HTL they may include inner hair cells and neural processes. If this was the case, a low to moderate correlation was expected between OAE and HTL.

There have been many experimental studies investigating the link between OAE and HTL in human subjects, but most have shown only a weak to moderate relationship between OAE and HTL (e.g. Gorga et al, 1993 a, b). Although different OAE parameters have been studied, most research has concentrated on OAE amplitude obtained using high stimulus levels. Recently, measures of OAE that take into account the level dependency of the cochlear amplifier have been described (Mills, 1997; Dorn et al, 2001), and these were thought to have a higher correlation with HTL. Measures that enable recording to low noise floor are proposed to improve the correlation with HTL. Also most experimental studies have been cross-sectional, and the moderate correlations of previous studies may be a result of inter-subject and inter-ear differences, such as middle ear factors influencing the relationship between OAE and HTL

This thesis aimed to address these points, and to test two hypotheses. Firstly it was hypothesised that the moderate correlation between OAE and HTL shown in previous studies is a result of a poor choice of OAE measures. It was expected that OAE measures that account for the level dependency of the cochlear amplifier (CA) would show a higher correlation with HTL than previous measures. Secondly it was hypothesised that that the moderate correlation between OAE and HTL arises from inter-subject and inter-ear differences that influence OAE and HTL differently. It was expected that longitudinal changes in OAE within subjects would show a higher correlation with HTL than cross-sectional differences.

These hypotheses were tested in two experiments: a cross-sectional experiment investigating differences between OAE measures across subjects with a range of HTL (up to mild/moderate sensorineural hearing loss), and a longitudinal experiment investigating changes in OAE measures

within subjects undergoing a temporary hearing threshold shift from salicylate. A range of OAE measures were investigated. Equipment with low noise floor was used, and high stimulus rates were employed, with the aim of recording OAE to low levels. OAE measures were based on published models, including OAE input/output (I/O) functions based on Mills model (1997) of DPOAE in small mammals, and TEOAE rate suppression based on the model of TEOAE I/O nonlinearity Kapadia and Lutman (2001). Both distortion product (DP) and transient evoked (TE) OAE were measured across a range of stimulus levels and stimulus rates, taking account of cochlear fine structure.

The cross-sectional experiment measured a range of OAE. The maximum length sequence (MLS) technique for measuring TEOAE at increased click rates enabled recording down to a noise floor of approximately –25 dB SPL, compared to approximately –10 dB dB SPL with the conventional method. The DPOAE equipment also allowed recording down to a noise floor of approximately –30 dB SPL at the high frequencies. OAE measures were evaluated by the strength of their correlation with HTL. There was a higher correlation between OAE and HTL when lower level stimuli were used, although the relationship was still only moderately close, with maximum correlation coefficients of –0.7 to –0.8 at some frequencies. In general DPOAE measures had higher correlations with HTL than TEOAE. TEOAE measured using the MLS recording technique at higher click rates did not show improved correlations with HTL. MLS TEOAE rate suppression measures showed only a weakly significant relationship with HTL.

Comparison of the correlation results of this study with other cross-sectional studies showed similar values. Pienkowski and Kunov (2001) used DPOAE suppression tuning curves and related the measure to HTL. The maximum correlation they reported was 0.7 at 4 kHz. Dorn et al (2001) used DPOAE I/O functions to calculate DPOAE threshold and related this to HTL, with a maximum reported correlation of 0.86. Therefore, the hypothesis that new OAE measures investigated in this thesis would have an increased correlation with HTL was not upheld.

The longitudinal experiment examined whether inter-subject and inter-ear differences were influencing the relationship by investigating changes in OAE within subjects, and their relationship with HTL. Salicylate was used to induce a temporary hearing threshold shift in a group of normal hearing subjects. Changes in OAE and HTL were investigated. Subjects showed a range of hearing threshold shifts, up to a maximum of 20 dB, and a range of OAE changes, up to 15 dB for DPOAE and 10 dB for TEOAE. The correlation of the group changes in OAE and HTL showed only a moderate relationship, and the values were no higher than the correlations measured in the cross-sectional experiment. This was observed for all the OAE measures used.

However investigation of individual subjects showed that some subjects and some ears had highly significant correlations between the change in OAE and the change in HTL over the course of the seven test sessions. The correlations between OAE and HTL in these subjects and ears were higher

than the cross-sectional differences. Approximately 60% of ears showed significant relationship between DPOAE and HTL, varying across a range of frequencies, and varying in the number of correlations. For TEOAE and MLS TEOAE the figure was 50% of ears. For MLS rate suppression the figure was 30%.

Based on the group results of the longitudinal study, the hypothesis that changes in OAE would show higher correlations with HTL than cross-sectional differences was not upheld. This was observed for all OAE measures. However for approximately 50% of ears, there were highly significant correlations between the changes in OAE and HTL that were higher than the cross-sectional results. This was found in decreasing numbers of ears for DP, TEOAE and MLS TEOAE rate suppression. There was a wide range of frequency relationships between the OAE and HTL measures, and in those subjects with a high number of correlations between the change in HTL and OAE, the relationship was not always frequency specific. This was observed for both DP and TEOAE. For these ears the hypothesis holds, and suggests that inter-ear differences are responsible for some of the variation in the relationship between OAE and HTL across subjects.

From the results of the two experiments, it seems that the main assumption of the thesis, that there is a close relationship between OAE and HTL mediated through the CA, is false. Although it was a simplistic assumption, the fact that it was upheld for some ears implies that it was a useful starting point and is appropriate for a percentage of cases. However to explain the results of the whole group, other factors responsible for OAE generation and sensory hearing threshold also need to be taken into account. This is shown diagrammatically in Figure 8-1. This diagram shows that to describe the population, a variety of factors must be considered. However within the population a group of ears can be described by a simple model in which OAE and HTL are closely related; this is likely to be through the OHC and the cochlear amplification mechanism. However this relationship is both level and frequency dependent, and work is required to investigate this further. The rest of the population requires a complicated model, in which OAE and HTL are not closely linked. It is likely that other factors for OAE generation and sensory hearing threshold are important in this group. It was assumed that differences/changes in sensory hearing threshold level, over the range investigated in this thesis were due to OHC dysfunction only. Inner hair cells and neural processes may have had an influence on hearing threshold, and are included in the relationship. The relationship of OAE with HTL is also likely to be influenced by other factors involved in OAE generation apart from the CA. For example, minor changes to reflection sites within the cochlea may lead to a change in OAE level but with no associated change in HTL. For this thesis, the efferent system was included within the overall concept of the CA. For future work, it may be appropriate to investigate the influence this has on the variation in OAE and HTL.

The framework from which the OAE measures were derived was based on published models of OAE. These included OAE I/O functions based on Mills model (1997) of DPOAE I/O functions in small

mammals. The results of this thesis suggest that this model requires modification for transfer to human subjects. The model was based on the concept of active and passive DPOAE, and recent work has shown this concept is outdated (Mills, 2002). Although there was a general trend of a reduction in low level DPOAE and increased linearity of I/O functions with both differences and changes in HTL, the relationship was not consistent across subjects. For models of DPOAE in human subjects it may be appropriate to include other DPOAE generation sites as well as the f_2 site, and also to include other DPOAE generation mechanisms as well as CA gain.

It was not possible to transfer Mills model of DPOAE I/O functions to TEOAE I/O functions in humans. Although the broadband TEOAE responses were similar to the predicted framework, the frequency-banded responses did not show an increase in linearity or a disproportionate reduction in low level TEOAE with differences or changes in HTL. This is likely to be related to the different generation mechanisms of TEOAE compared to DPOAE, and possibly the greater reflection component of TEOAE.

The results of this thesis show that DPOAE have advantages over TEOAE for investigating auditory sensitivity. Although further work and experimentation is required, DPOAE have higher correlations with HTL, both between and within subjects and ears.

A further original investigation of the thesis was evaluation of the theoretical model relating MLS rate suppression and TEOAE I/O function nonlinearity as proposed by Kapadia and Lutman (2001). The results of this thesis showed a close relationship between rate suppression and the nonlinearity of the I/O function. This provides evidence for a cochlear mechanism for MLS rate suppression, particularly at the high frequencies.

Shera and Guinan (1999) proposed that TEOAE would be more sensitive to salicylate than DPOAE. However the results of this study showed no evidence of this. Comparison of the relationship between DP and TEOAE showed the highest correlation between the OAE types at the mid-frequencies, and at low stimulus levels. The relationship between DP and TEOAE was improved after consumption of salicylate at some frequencies. This may be due to a change in the reflection or distortion components of DPOAE making it more like TEOAE. Knight and Kemp (1999) showed that the $2f_2 - f_1$ DPOAE is more closely related to TEOAE than the $2f_1 - f_2$ DPOAE. They postulate that this is due to the greater reflection component of the $2f_2 - f_1$ DPOAE, making it more like TEOAE than the $2f_1 - f_2$ DPOAE. Salicylate may therefore be increasing the proportion of reflection to distortion mechanisms in DPOAE generation in this experiment, making it more like TEAOE. It would be helpful to examine phase changes to understand these changes in OAE generation.

The limitations of this experiment were that the hearing losses were small and occurred over a relatively short period of time. Further experiments should measure OAE phase to differentiate between changes in reflection or distortion mechanisms of generation. Further investigation is

required as to why some subjects showed good correlations between OAE and HTL whereas others did not, and also to investigate the relationship of OAE measures with frequency.

To summarise, the main premise of the thesis, that there is a close relationship between OAE and HTL, mediated through the CA, is not upheld. Although it was appropriate for a percentage of cases, to explain the results of the whole group, it seems likely that other factors responsible for OAE generation and sensory hearing threshold are important. Further work is required to investigate these factors. Further work is also required to test the predicted relationship of the change in OAE with the change in HTL and to determine the individual ear/subject factors that affect the relationship.

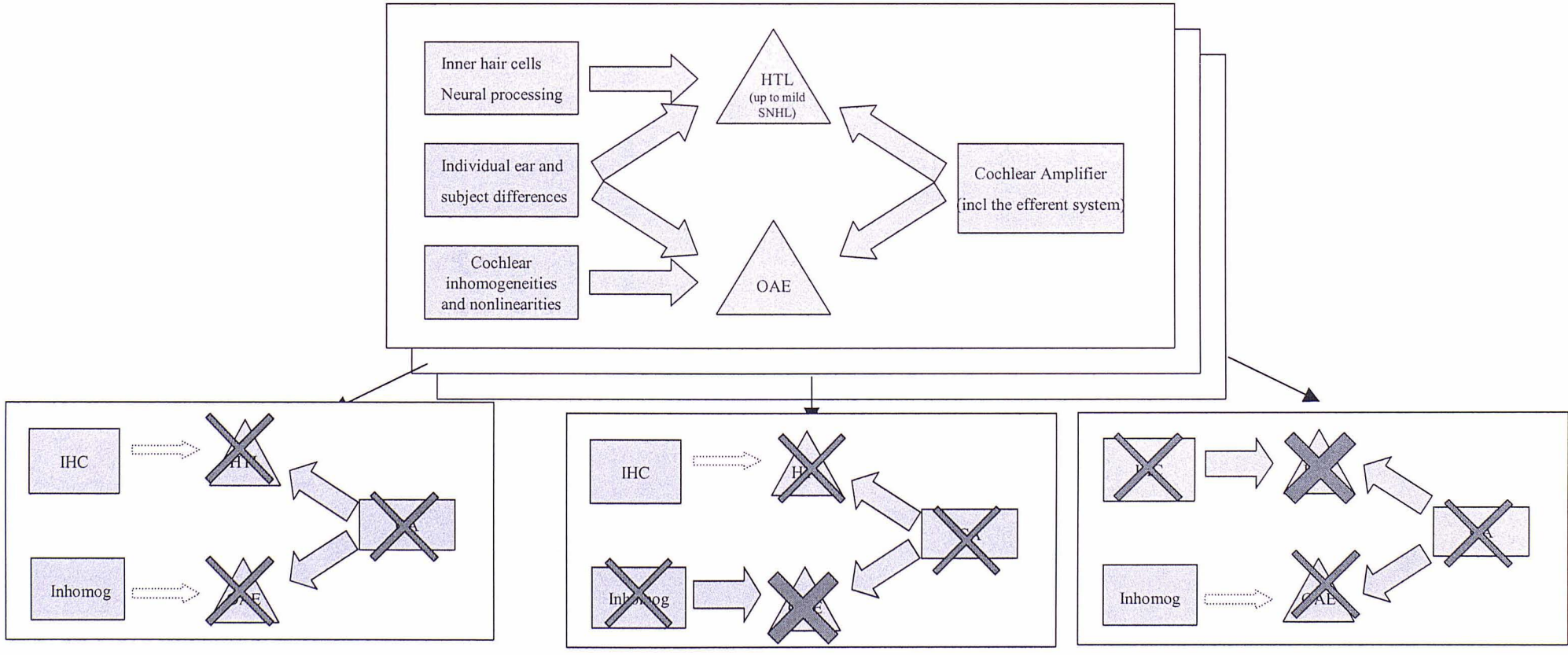


Figure 8-1: Update of the conceptual diagram of the relationship between hearing threshold and otoacoustic emissions, based on the results of this thesis. A) This shows the factors that are likely to describe the relationship for the whole population. It includes both cochlear amplification and other factors as important in the relationship between OAE and HTL. B) to D) give examples of the different types of relationship measured between OAE and HTL with salicylate consumption for different ears. Possible reasons for the different types of relationship are given. B) In this example there is a high correlation between HTL and OAE, mediated through the CA. A reduction in cochlear amplification affects HTL and OAE equally. C) There is a weak relationship between HTL and OAE. A reduction in cochlear amplification affects HTL and OAE, however there are other influences on OAE generation. D) There is a weak relationship between HTL and OAE. A reduction in cochlear amplification affects HTL and OAE, however there are other influences on HTL. This diagram does not show the effects of frequency or the different types of OAE. Key to abbreviations – CA: cochlear amplifier, IHC: inner hair cells, OAE: otoacoustic emissions, SNHL: sensorineural hearing loss

Appendix 1: Proof of cubic growth of DPOAE

For a system with two inputs f_1 and f_2, as is the case for DPOAE, the cubic component of the power series is the distortion product $2f_1-f_2$. f_1 is represented here by x_1 and f_2 by x_2.

For two input waveforms (f_1 and f_2)

$$x(t) = x_1(t) + x_2(t) \qquad \textit{(Equation 1)}$$

where

$$x_1 = A_1 \sin 2\pi f t \qquad \textit{(Equation 2)}$$

$$x_2 = A_2 \sin 2\pi f t \qquad \textit{(Equation 3)}$$

thus

$$x(t) = A_1 \sin 2\pi f t + A_2 \sin 2\pi f t \qquad \textit{(Equation 4)}$$

where

A = amplitude

f = frequency

t = time

The output due to the cubic term a_3x^3 is

$$a_3 (A_1 \sin\omega_1 t + A_2 \sin\omega_2 t)3$$

$$= a_3 A_1^3 \sin^3\omega_1 t + a_3 A_2^3 \sin^3\omega_2 t$$

$$+ 3a_3 A_1^2 \sin^2\omega_1 t\, A_2 \sin\omega_2 t + 3a_3 A_1 \sin\omega_1 t A_2^2 \sin^2\omega_2 t \qquad \textit{(Equation 5)}$$

where

$\omega = 2\pi f$

Expanding the third term of the right hand side of the equation gives the distortion product $2f_1-f_2$ as follows:

$$3a_3 A_1^2 A_2 \sin^2\omega_1 t\, A_2 \sin\omega_2 t$$

$$= \frac{3}{2} a_3 A_1^2 A_2 (1 - \cos 2\omega_1 t) \sin\omega_2 t \qquad \textit{(Equation 6)}$$

$$= \frac{3}{2} a_3 A_1^2 A_2 \sin\omega_2 t \quad - \frac{3}{4} a_3 A_1^2 A_2 [\sin(2\omega_1+\omega_2)t \; - \mathbf{sin(2\omega_1-\omega_2)t}]$$

The $2f_1-f_2$ component is highlighted in bold.

The growth of the cubic component $2f_1-f_2$ with stimulus level is 3 dB/dB. The amplitude of the $2f_1-f_2$ is represented by

$$\frac{3}{4} a_3 A_1^2 A_2 \qquad \textit{(Equation 7)}$$

The amplitude of $2f_1-f_2$ is proportional to the amplitude of f_2 and to the square of the amplitude of f_1. When $A_1=A_2$ (ie. L1=L2), this can be represented as

$$\frac{3}{4} a_3 A_1^3 \qquad \textit{(Equation 8)}$$

The amplitude of $2f_1-f_2$ therefore has a cubic relationship with the amplitude of the input stimulus. When the I/O function of this relationship is plotted on a log-log scale, the function will have a slope of 3.

This is also true when $A_1 \neq A_2$ (L1 $\neq$ L2) as long as the difference between them remains constant. This is shown below

if

$$A_1 = pA_2 \qquad \textit{(Equation 9)}$$

where

p=the constant difference

and the amplitude of $2f_1-f_2$ is represented as

$$A_1^2 A_2 \qquad \textit{(Equation 10)}$$

then

$$A_1^2 A_2 = p^2 A_2^2 A_2 \qquad \textit{(Equation 11)}$$

$$=p^2 A_2^3$$

The cubic relationship with input amplitude level is still maintained as long as p is preserved as a constant.

Appendix 2: Calibration section

SELF RECORDING AUDIOMETRY

Equipment

Type 1 sound level meter and filter set

Pistonphone sound calibrator

IEC 303 acoustic reference coupler and one inch microphone

Sessional Calibration

1. Carry out a subjective listening check across the frequency range.
2. With a 100 dB output on the computer, and 30 dB on the audiometer, set the green LED display of the audiometer to 0.

Weekly Calibration

1. Place the left headphone on the IEC 303 coupler.
2. Load the Self Recording Audiometry software and then generate a continuous tone from the computer by selecting *Manual*. Use the following parameter settings:

   Level **100**

   Frequency step **50**

   Pulses per frequency **1000**

   Pulses per level **1000**

   Start level **100**

   Level step **2**

   Inter pulse interval **1500**

   Duration **1400**

   Onset/offset **20**

   Envelope shape **linear**

3. Set the audiometer to Left headphone, CD1 and a level of 30 dB HL. With the self recording audiometry parameters as above, set the LED display to 0 by altering the knob above the CD1 button.
4. Measure the output of the headphones in dB SPL at test frequencies of 2, 3, 4, 5 and 6 kHz. Convert these values to dB HL using the RETSPL values given in ISO 389.

Monthly Calibration

1. As for the weekly calibration but also measure the intermediate frequencies in dB SPL at each 50 Hz step from 2500 to 6500 Hz.
2. Figure 8-2 plots the RETSPL as given in ISO 389. Interpolate the RETSPL values for the intermediate frequencies and convert to dB HL.

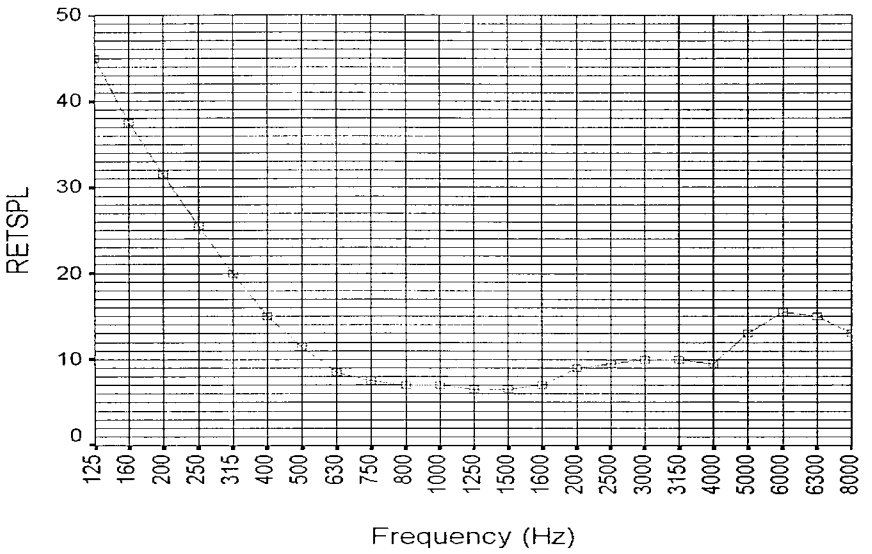


Figure 8-2: RETSPL values for intermediate audiometric frequencies (derived from ISO 389)

TRANSIENT-EVOKED OTOACOUSTIC EMISSIONS (ILO 88 v.4.20)

Equipment required

- Type 1 sound level meter and filter set
- Pistonphone sound calibrator
- Zwislocki coupler and one inch microphone
- 1 kHz tone generator (audiometer)

Sessional Calibration

1. Run the probe cavity test (menu 1, return rather than go after check-fit gives the option), as per ILO manual. Check the following measurements:

A+B	≥ 15 dB
A-B	≤ 3 dB
Reproducibility overall	> 90 %
Reproducibility at 1 kHz	> 90 %
Reproducibility at 4 kHz	> 90 %
Stability	> 90 %

Compare the screen print to the factory printout.

2. Record an emission with the probe in your own ear using the nonlinear mode to verify the presence of an OAE.

Weekly Calibration

A. Microphone sensitivity

1. Using menu 2, *Change Stimulus Level* and set gain to OFF to turn off the click and the reference stimulus.
2. Connect the probe to the Zwislocki coupler, and introduce a 1 kHz continuous tone to the coupler using the earphone tube attachment to give approximately 60 dB SPL. Measure the intensity on the SLM (1 kHz fast ext.filt).
3. Run the ILO only in check-fit mode and observe the Reference Stimulus window on the ILO screen. Record to the nearest 1 dB the peak that you see at 1 kHz. Check that a pure tone (sine wave) is displayed in the other two ILO stimulus windows.
4. Repeat for a 1 kHz tone using a dial setting of 10 dB less. The displayed level should be within 3 dB of the SLM level.
5. Repeat the above with pure tones of 2 and 4 kHz.

B. Click sensitivity

1. Attach the Zwislocki coupler and microphone to the SLM and connect a storage oscilloscope to the output from the SLM. Adjust the SLM using a sound calibrator in the usual way. Apply a continuous 1 kHz tone from the audiometer to the Zwislocki coupler using the insert earphones, and adjust its amplitude to achieve a reading of 70 dB to the nearest 2.5 dB as measured at the 1" microphone. Note the peak-to-peak reading on the oscilloscope (1 kHz fast. ext filt) and the actual rms sound pressure level measured. This effectively calibrates the oscilloscope graticule in terms of sound pressure.

2. Attach the ILO probe to the Zwislocki coupler using the modified GSI probe. Run the ILO in it's preset mode and with the click amplitude gain at it's default of 0 dB. Set the SLM to a range of 3.3 - 83.3 dB. On the oscilloscope you will see groups of 4 clicks, 3 of which are at a lower intensity than the 4th. Measure the peak-to-peak reading on the oscilloscope of the higher and lower level clicks. Calculate the peak equivalent SPL of each to the nearest 0.5 dB. Try to avoid altering the voltage scale on the oscilloscope. (Note: The peak equivalent SPL of a transient sound is the RMS SPL of a steady pure tone which has the same peak-to-peak amplitude.)
3. Run the ILO set to linear click. Continue with the gain set at it's default of 0 dB. Measure the peak-to-peak reading on the oscilloscope and calculate the pe SPL of the linear clicks.
4. Using menu 2, *Change Stimulus Level* to alter the gain to -10.5 dB (for both the test clicks and the reference clicks), run the ILO set to linear click again. Alter the output gain on SLM/measuring amplifier if necessary and repeat the peak-to-peak measurement.
5. Repeat for clicks with a gain of - 21.0 dB. Remember to account for any alterations to the SLM when calculating the peak equivalent SPL. Check that the difference in SPL measuredis within 1.0 dB of the nominal difference. (Note: The oscilloscope trace for click gain -21.0 dB will be noisy. The objective is to measure to an accuracy of +/- 10 %.)

TRANSIENT-EVOKED OTOACOUSTIC EMISSIONS (MLS Natus)

Use the same method as for the ILO288, with the click opportunity rate set to 50 click/s.

DISTORTION PRODUCT OTOACOUSTIC EMISSIONS

Notes on calibration of DPOAE

Calibration of the DPOAE measurement system is discussed here as this has bearing on the analysis and interpretation of results, and also when comparing results with other published papers.

Calibration of DPOAE probes must consider the effect of standing waves from the primary stimulus tones in the ear canal. Standing waves result from the interaction of reflected waves from the eardrum with the incident stimulus wave. When incident and reflected waves interact in phase, the waveforms summate to give an overall increase in ear canal SPL. When they interact out of phase, the waveforms cancel each other out to give a reduction in ear canal SPL.

OAE probes are designed so that the probe microphone port is approximately 15-20 mm from the eardrum. This microphone may be used to measure the SPL of the primaries in the ear canal. However, this can lead to errors in the estimation of the SPL at the eardrum, particularly at high frequencies due to the problem of standing waves. The distance of the probe microphone from the

eardrum results in cancellation of waveforms at the probe microphone (but not at the eardrum) most markedly at frequencies of 5-7 kHz (Siegel and Hirohata, 1994) but to some extent above approximately 3 kHz. This can lead to a SPL reduction of between 10-20 dB at the microphone although not at the eardrum (Whitehead et al, 1995). This effect is variable amongst ears and unpredictable in detail for any individual. The ideal solution to measure SPL correctly at the eardrum would be to use a probe microphone at the eardrum. However this is not practical in most settings. These problems were considered when deciding the method for calibration of both the OAE probe earphones and microphone. In brief, it was decided not to use the probe microphone to set the primary levels individually for each ear, but to use a calibrated driving voltage to the earphones. Nonetheless, standing waves can also affect calibration in a coupler and this situation was considered in the calibration of the probe microphone described below.

Earphone calibration:

There are three different methods that have been used to calibrate OAE probe earphones. The first uses an in-the-ear adjustment strategy, which alters the voltage to each earphone as a function of frequency, to produce a constant SPL at the DPOAE probe microphone. Some commercially available DP measurement systems use this method (Whitehead et al, 1995). This method has disadvantages because it makes a correction to the earphone output based on the measurement at the probe microphone, which deviates from the level at the eardrum. At frequencies above 3 kHz, standing waves result in some cancellation at the microphone but not at the eardrum[3]. This method wrongly makes a correction for this cancellation, which results in an artefactual increase in stimulus SPL at the eardrum.

The second and third methods are based on the frequency response of the ER-2 earphone, which is relatively flat when measured in a coupler. The implication is that this will also be the case at the eardrum. The second method thus uses a constant voltage as a function of frequency to produce an assumed SPL at the eardrum (Whitehead et al, 1995). The constant-voltage method does not over-compensate the stimulus level in the same way the in-the-ear method. However, it is still influenced by inter-subject variability in ear canal size and geometry.

The third method is also based on the constant voltage method, but does not assume a completely flat frequency response, as this is unlikely to be the case where deviations across frequency can be up to 10 dB. Frequency response is measured in an ear simulator and corrections made to the output if

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[3] At frequencies above approximately 7 kHz the probe microphone may be in the vicinity of an antinode in the standing wave pattern. Therefore the logic applying to lower frequencies is reversed and minor under-compensation may occur, although this cannot exceed 6 dB for theoretical reasons.

necessary. This is the preferred method and was the method used to calibrate the DPOAE earphones in this thesis. See Appendix 2 for detailed description of the calibration method.

A fourth method might be to correct for the individual ear canal, based on probe microphone measurement at 1 kHz then assuming this correction applies at all frequencies (plus corrections for method three).

Microphone calibration:

There are two methods for calibrating the DPOAE microphone. The first method is based on the relatively flat frequency response curve of the ER-10B microphone. The assumption can be safely made that no adjustment to microphone frequency response is required and that the same sensitivity can be applied across all frequencies. Sensitivity is set at 1 kHz, and this calibration value is then applied to all frequencies.

The second method is described by Harris et al (1989). They adjusted the level of their DPOAE data based on the fact that the output of the microphone to a constant voltage single-tone input was not flat. Their method aimed to compensate for standing wave cancellation at the probe microphone from the stimuli generated at the probe, which were reflected by the eardrum. However this could lead to errors, as this cancellation at the probe microphone does not occur for DPOAE, which are generated at the eardrum (and possibly reflected at the probe). Adjusting DPOAE level based on this assumption will lead to errors in estimation of DP level (Whitehead et al, 1995).

Method one was used to calibrate the DPOAE probe microphone in this thesis for the reasons discussed above.

Equipment

- Type 1 sound level meter and filter set
- Pistonphone sound calibrator
- Zwislocki coupler and microphone

Sessional Calibration

Run a single distortion product measure with the probe in your own ear to verify the presence of a DPOAE.

Weekly/monthly Calibration

A. Tone sensitivity

1. Attach the Zwislocki coupler and one inch microphone to the SLM. Calibrate the SLM using the piston phone in the usual way. Then set the SLM to spectrum analysis on a fast setting.
2. Attach the DPOAE probe to the coupler using a probe tip.

3. Run the DPOAE software. Use the *Settings* menu, and select *Calibration* function. From this screen, both the earphones and the microphone can be calibrated. Alter the *Measurement Parameters* at the bottom of the screen if necessary:

   Nominal level **50 dB** (output level of the earphone)

   Buffers **100**

   Rejection level **10 dB**

4. Select Earphone A. Highlight the frequency to be calibrated, make sure this is done for both the microphone correction and earphone correction tables. Press *Play*. This plays the tonal frequency of choice at the selected nominal level.

5. Measure the level on the SLM, it should read 50 dB SPL (or whatever the nominal level is set to). If the level deviates from 50 dB, alter the earphone correction factor for this frequency by the required amount. Press *Play*, and remeasure the earphone output. This should now read 50 dB.

6. Repeat the procedure for each frequency. When each frequency has been measured, press *OK*, which will save any changes made.

7. Select Earphone B, and repeat the procedure for the second earphone.

B. Microphone sensitivity

1. When the earphone output has been calibrated, set the frequency to 1 kHz and play a level of 50 dB. Read the *microphone level* from the top left corner of the screen. This should also be 50 dB. If the microphone level deviates from 50 dB, alter the microphone correction factor by the required amount. Press *Play* and remeasure the microphone level, which should now read 50 dB.

2. Adjust the microphone sensitivity across the frequency range to the level set at 1 kHz.

Appendix 3: Fast Fourier Transform methods

This section describes the Fast Fourier Transform (FFT) analysis applied to the TEOAE and MLS TEOAE results. This formed a substantial part of the developmental work.

The analysis was performed in Microsoft Excel using the FFT analysis tools to validate the on-screen analysis of the ILO288 software, and to ensure consistency of FFT analysis methods with the MLS TEOAE data. Following validation of the ILO analysis, the majority of further analysis was performed using the ILO on-screen frequency analysis tools. The validation method was informed by that of Haughton (1998), who described the Excel functions that can be used to reproduce the ILO on screen analysis values. Whilst the principles involved are standard, the detailed implementation was important for comparison of the methods performed by the ILO288 and the MLS Natus system, and with other studies.

FAST FOURIER TRANSFORM ANALYSIS

Fourier transform allows time series data (x_t) to be approximated by a sum of sine and cosine terms, called the Fourier series representation (Equation 12) (Chatfield, 1995)

$$x_t = a_0 + \sum_{p=1}^{(N/2)-1} [a_p \cos(2\pi pt/N) + b_p \sin(2\pi pt/N)] + a_{N/2} \cos\pi t \qquad \textit{(Equation 12)}$$

where t = 1, 2 .... N

and N = sample number

From Equation 12 the time series data can be simplistically represented as the sum of a series of sine and cosine waveforms.

$$x_t = a_0 + b_0 + a_1 + b_1 \ldots a_N + b_N \qquad \textit{(Equation 13)}$$

where a represents cosine and b sine.

The Fourier coefficients are represented by a_p and b_p where

$$a_p = 2\,[\Sigma x_t \cos(2\pi pt/N)]/N \qquad \textit{(Equation 14)}$$

$$b_p = 2\,[\Sigma x_t \sin(2\pi pt/N)]/N \qquad \textit{(Equation 15)}$$

for p = 1, 2 .... (N/2) - 1

The effect of the Fourier coefficients within the Fourier series representation is to multiply the time series data by each coefficient (as a function of p) within the Fourier series. This identifies any component of the waveform at the same frequency as the coefficient, thus breaking down the waveform into its individual constituents, in terms of frequency and amplitude.

Amplitude of the *p*th harmonic is then calculated as

$$(a_p^2 + b_p^2) \qquad \textit{(Equation 16)}$$

Fast fourier transform is a quicker, more accurate method to perform a Fourier transform and employs complex numbers for mathematical simplicity. In an FFT, the Fourier coefficients (Equations 14 and 15) are represented as:

$$a_p + ib_p = 2\,[\Sigma x_t\, e^{2\pi ipt/N}]/N \qquad \textit{(Equation 17)}$$

for p = 1, 2 .... (N/2) - 1

To optimise the calculations within the FFT, it is preferable that N is highly composite, of the form 2^n. If the waveform is not in this format, then the data should be zero-padded to increase N to a suitable integer. If zero-padding is used, then the data may require windowing to avoid discontinuity errors. The remaining calculations are as described above.

FFT ANALYSIS OF THE ILO288 WAVEFORM DATA

The ILO288 stores the average microphone signal in alternate buffers A and B. Prior to display, the responses are windowed and filtered. This includes high-pass filtering at 200 Hz to remove some of the stimulus artefact. Responses are then windowed using a cosine ramp, with rise and fall times of 2.56 ms. The initial 2.5 ms of the time window are zero-padded. After both these procedures, the response is forward and reverse bandpass filtered between 0.6 and 6 kHz. This process estimates any delay or phase shift from filtering. The relevant parameter settings of the ILO288 are shown in Table 8-1.

Table 8-1: ILO288 parameter settings

Parameter	Value
Time window (ms)	20.44
Response window (ms)	2.5 to 20.44
Sample number	512
Sample rate (Hz)	25000
Sampling interval (μs)	40
Frequency resolution (Hz) (sample rate/sample number)	48.83

The average waveforms at this stage can be output in ASCII-file format for the A and B buffers. Each waveform is output as a total of 512 points, which can then be analysed externally. Alternatively, the data can be analysed using the in-built tools within the ILO288 software.

The ILO data output was analysed in Excel using the FFT analysis tools function. The following method was used:

1. The ASCII file output of the A and B waveforms were transferred to Excel. The two waveforms obtained to a click level of n dB are denoted *a* and *b*, where *a* and *b* are vectors of length 512. The ILO288 output file contains integer values scaled in μPa.

2. The difference between the waveforms *a* and *b* is a measure of the noise.

   Noise (*x*) was calculated using the formula as described in the ILO288 user manual.

   $$x = (a-b)/\sqrt{2} \qquad \textit{(Equation 18)}$$

3. Waveforms *a*, *b* and *x* underwent FFT analysis using the Fourier analysis function in the Data Analysis tools menu, where A and B are complex vectors of length 257.

   $$A=fft(a) \qquad \textit{(Equation 19)}$$

   $$B=fft(b) \qquad \textit{(Equation 20)}$$

   $$X=fft(x) \qquad \textit{(Equation 21)}$$

4. These values were used to calculate the cross-power spectrum and also analyse into 1/6-octave and octave frequency bands. The cross-power spectrum (*C*) and noise spectrum (*N*), which were scaled in decibels were calculated by:

$$C = 10\log10(XX^*) \quad \textit{(Equation 22)}$$

$$N = 10\log10(AB^*) \quad \textit{(Equation 23}$$

where

A^* is the complex conjugate of A

B^* is the complex conjugate of B

X^* is the complex conjugate of X

The data were analysed into 1/6-octave frequency bands by summating the power (i.e. AB^* or XX^*) over the frequency range in Table 8-2. The summated values may be represented by C_S. These were then converted to decibels (peak amplitude values) using the formula:

$$10\log_{10}(Cs^*) \quad \textit{(Equation 24)}$$

Table 8-2: Frequency range used in TEOAE analysis

Bandwidth (octave)	Frequency (kHz)	Frequency range (Hz)	FFT points
Broadband		0-6250	1-129
1/6	1	878-1123	19-24
	2	1757-2246	37-47
	3	2685-3320	56-69
	4	3564-4443	74-92
	5	4443-5566	92-115
	6	5322-6689	110-138

5. The spectrum values and the frequency band analysis were scaled to achieve values in dB SPL. This was on account of:

   a. Scaling for the number of points in the FFT

$$-20\log512 = -54.185 \text{ dB} \quad \textit{(Equation 25)}$$

b. Referencing to sound pressure at 20 μPa

$-20 \log 20 = -26.021$ *(Equation 26)*

c. Conversion from rms to peak sound pressure level

$+20 \log 0.707 = +3.010$ dB *(Equation 27)*

FFT ANALYSIS OF THE ILO288 WAVEFORM DATA

The MLS Natus machine stores the averaged microphone signal in alternate buffers A and B. The averaged waveforms at this stage can be output in ASCII-file format for A and B buffers. Each waveform is output as a total of 357 points, which corresponds to a time period of 5.0376 to 17.0136 ms. It was first necessary to determine the scaling of the waveform data. Due to the developmental nature of the device there was no manual and little available information regarding the format of the output data. It was therefore necessary to validate the scale of the waveforms, thought to be μPa. The MLS Natus software was limited in frequency analysis tools. The software generated the TEOAE signal-to-noise ratio at octave frequency bands, but performed no other frequency analysis. There were no other facilities for further analysis of the data. It was therefore necessary to validate the limited processing performed by the software and to develop a method to analyse the MLS data comparable to the ILO288. The relevant parameter settings of the MLS Natus machine are shown in Table 8-3.

Table 8-3: MLS Natus parameter settings

Parameter	Value
Time window (ms)	17
Response window (ms)	5 to 17
Sample number	510
Sample rate (Hz)	29976.02
Sampling interval (μs)	
Frequency resolution (Hz) (sample rate/sample number)	58.77

The FFT analysis was performed in Excel using the following method:

1. The ASCII file output of the A and B waveforms were transferred to Excel. The two waveforms obtained to a click level of n dB are denoted *a* and *b*, where *a* and *b* are vectors of length 357.
2. The waveforms were windowed to smooth the onset and offset of the response. A cosine window was used (rise/fall time of 2.5ms) with a plateau between these points. The window formula was

$$1/2\ (1 + \cos\ (2\pi n/N)) \qquad \textit{(Equation 28)}$$

where N = total number of points over the range and n= –N/2 to N/2

The shape of the cosine window is shown in Figure 8-3.

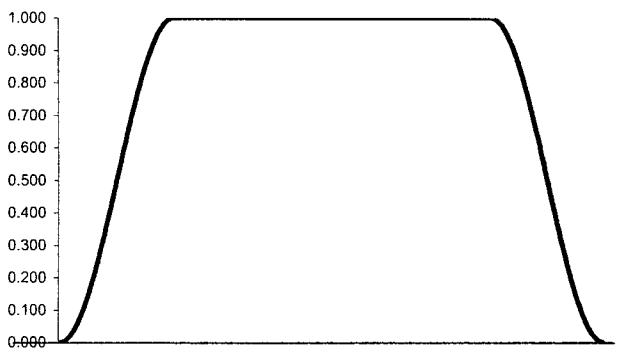


Figure 8-3: Cosine window with a 2.5 ms rise/fall time

3. The ASCII file contained 357 data points while the Excel FFT analysis tool requires the number of input values to be a power of 2. Therefore the data points 1 to 155 were zero padded to give a total of 512 points.
4. To correct for the 5 dB reduction in intensity that occurred from the padding and windowing process, the a and b waveforms were divided by a factor of 0.554688. The waveforms were then analysed using the methods described for the ILO waveforms (number 2 onwards).
5. The data were analysed into 1/6-octave frequency bands by summating the power (i.e. AB* or XX*) over the frequency range shown in Table 8-4.

Table 8-4: Frequency range used in MLS TEOAE analysis

Bandwidth (octave)	Frequency (kHz)	Frequency range (Hz)	Waveform points
Broadband			1-126
1/6	1	878-1112	15-19
	2	1756-2224	30-38
	3	2693-3337	46-57
	4	3571-4449	61-76
	5	4449-5562	76-95
	6	5328-6674	91-114

6. Comparison of the calculated FFT spectrum with the Natus display screen showed them to be similar. However in terms of amplitude there was a difference of approximately 14 dB between the Natus display screen and the Excel calculations (the Natus FFT was larger). This was due to averaging in 58 Hz blocks.

Appendix 4: Audiological screening questionnaire

NAME_____**dob**_____

Address_____

Do you suffer from any of the following:

Hearing problems.....

Tinnitus.....

Current ear disease (e.g. persistent ear pain, ear infection or ear discharge) .....

Vertigo or dizziness.....

Cardiovascular disease .....

Epilepsy.....

A psychiatric condition .....

Other (please specify) .....

Are you on any medication? .....

Noise exposure:

Have you ever been exposed to noise at work? .....

If Yes: what kind of noise.....

How much? .....Hours/Day

.....Days/Year

for.....Years

To be heard in this noise do people have to speak normally/loudly/very loudly?

Have you ever been exposed to the noise of guns? .....

If so, what type? .....

Approximately how many rounds have you been exposed to?.....

Have you ever been exposed to any other loud noise, bomb blasts, explosions, etc. which seemed to have some permanent or temporary effect on your hearing? (If Yes please give details)

.....

Have you been exposed to loud noise within the past 24 hours? (If Yes please give details)

.....

Appendix 5: Experiment 1 Individual subject hearing threshold level data

Subject no.	Ear (R=right, L=left)	Frequency (kHz)			
		3	4	6	3 – 6 average
1	R	8.0	2.5	22.9	11.1
2	L	21.9	25.6	36.4	28.0
3	L	18.9	37.6	61.4	39.3
4	L	19.0	28.9	38.6	28.9
5	L				
6	R				
7	R	-8.6	-0.5	4.2	-1.6
8	L	-1.4	13.3	12.9	8.3
9	L	17.3	27.6	36.6	27.2
10	R	-5.3	0.1	6.9	0.6
11	R	1.0	9.2	10.2	6.8
12	R	3.3	13.2	20.7	12.4
13	R	16.7	16.4	19.0	17.3
14	L	4.2	7.3	17.9	9.8
15	R	16.0	4.0	9.5	9.9
16	L	35.4	39.0	66.2	46.9
17	L	12.3	12.1	22.4	15.6
18	R	14.8	17.7	7.5	13.3
19	R	6.2	6.9	11.5	8.2
20	L	35.1	36.6	26.9	32.8
21	L	23.3	35.0	67.0	41.8
22	R	0.3	-6.4	2.0	-1.4
23	R	1.7	4.6	10.9	5.8
24	R	1.8	1.6	4.2	2.6
25	L	0.7	10.6	22.3	11.2
26	L	0.8	11.1	21.0	11.0
27	L	20.7	22.6	41.2	28.1
28	R	-1.5	-1.8	4.8	0.5
29	R	27.5	33.6	34.6	31.9
30	R	9.8	21.6	34.5	22.0
31	L	2.4	25.2	53.2	26.9
32	L	15.0	13.3	28.4	18.9
33	L	13.3	6.2	12.1	10.5
34	L	21.1	37.2	50.8	36.4
35	L	14.9	8.4	13.9	12.4
36	L	26.6	36.6	57.0	40.1
37	R	10.0	23.3	40.3	24.5
38	R	1.5	13.2	-2.5	4.1
39	R	17.3	17.6	32.4	22.4
40	L	14.1	14.4	28.7	19.1
41	R	32.3	60.6	60.4	51.1
42	L	18.4	43.1	50.3	37.2
43	R	35.6	49.3	64.0	49.6

Appendix 6: Experiment 2 Medical screening form

Name Dob Age

Do you have or have you ever had:

Recent illness	Recent surgery
Asthma	Anaemia
Indigestion/heartburn	Raised blood pressure
Jaundice	Gout
Renal disease	Epilepsy
Nasal polyps	Heart problems
Bleeding tendency	Diabetes
Ear symptoms – otalgia, otorrhoea	Haemophilia
Dizziness	Haematuria
Tinnitus	

Allergies

Has taking aspirin or other NSAIDs ever caused:

difficulty breathing	rhinitis (blocked or runny nose)
skin rash	swelling of the lips or face

Alcohol intake
Smoking Family history

Drug history
Do you take any of the following:

Painkillers e.g. Brufen, Neurofen, Voltarol
Antacids
Anticoagulants e.g. Warfarin
Antiepileptics e.g Phenytoin, Valproate
Steroids
Cytotoxics e.g. Methotrexate
Diuretics e.g. Acetazolomide, Spironolctone
Antiemetics e.g. Metoclopramide, Domperidone
Uricosurics e.g. Probenecid, Sulphinpyrazole

O/E

Anaemia	jaundice	clubbing	lymphadenopathy
Pulse		BP	
Breath sounds	vesicular	wheeze	nose
Ears			

Fit to take part in study ☐ Not fit to take part in study ☐

Avoid: Loud noise, alcohol, other pain killers, any other drugs OTC or prescribed

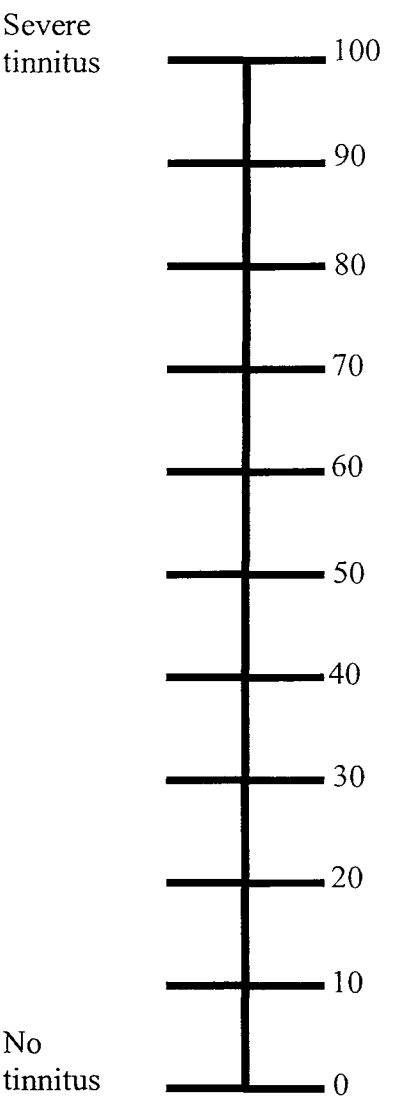
If experience: (nausea), abdo pain, GI or GU bleeding, tachychardia, sweating, dizziness, faint feeling, hyperventilation - STOP

Appendix 7: Experiment 2 Tinnitus rating scale

To help people state how good or bad their tinnitus, we have drawn a scale (rather like a thermometer) on which the absence of tinnitus is marked by 0 and severe tinnitus is marked by 100.

We would like you to indicate on this scale how good or bad is your tinnitus today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your tinnitus is today.

YOUR TINNITUS TODAY



Appendix 8: Experiment 2 Subject information sheet "Side-effects of aspirin"

Time commitment: You are required to attend a number of sessions over 7 days, 5 days of which are consecutive. For three of these days you are required to take aspirin tablets at set times. Each session lasts approximately 2 hours.

Your GP has been informed of your decision to take part in the project.

Aspirin: You have been given a total of 36 tablets and a dose box. There are 12 aspirin doses in total. Each aspirin dose comprises of 3 tablets. The aspirin dosage has been approved by the ISVR safety and ethics committee and is within the manufacturers maximum recommended dosage.

You should take 3 tablets with a glass of water and a small snack at each of the times stated on the timetable. You have been given a diary sheet to record the exact time of taking the tablets each day.

REMEMBER: If you are late taking a dose, leave 4 hours from that time until taking the next dose

Possible side-effects: As with most drugs, aspirin can produce side effects. Minor side-effects include tinnitus and mild, short-lived nausea. The risk of serious side effects from the dosage you will be taking is minimal, but you should be aware of the possible reactions. These include skin rash, gastro-intestinal haemorrhage, increased pulse rate, sweating, dizziness, hyperventilation, fainting. If you experience any of these, or if you are unsure, you should in the first instance contact Amanda Hall, either at the University (extension 22287, 023 80592287), or on her mobile (07881 665421). If the symptoms are such as to cause you worry (and in any case if you faint or experience sweating or increased pulse rate without obvious cause), then you should contact your GP and stop taking the aspirin immediately.

Blood sampling: On sessions 3, 4, 5 and 6 you will have a very small amount of blood taken. This will be done using an automatic thumb pricking machine.

Remember! During the study you should NOT:

- take any other medication
- drink alcohol, especially during the 3 days of aspirin consumptiongo to nightclubs, concerts or other places where you will be exposed to loud noise during the 3 days of aspirin consumption

Payment

- On completion of the experiment, you will be paid £100 for your trouble and expenses.
- You may withdraw at any time during the experiment without giving a reason.
- If you withdraw before the end of the experiment, you will be paid £10 per session completed.

Did you take the full dose of aspirin during the experiment?

(if no, why not and which doses did you not take?)

.....

Did you experience any side effects from the aspirin?

(if so, please state)

.....

.....

Did you seek medical help or advice during the course of the experiment?

(if yes, please give details)

.....

.....

Appendix 10: Experiment 2 Individual subject hearing threshold

Ear		L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Frequency (kHz)		1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4	4	4
Session number		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Subject number	1	0.6	2.1	0.0	-0.9	-1.1	-4.2	-3.2	1.2	-0.2	0.8	-0.6	1.9	-2.2	-2.9	-1.3	-1.3	-1.0	-0.5	-3.7	-6.4	-8.1	1.1	3.1	0.6	3.6	3.7	0.8	-0.9
	2	-3.0	-5.6	-5.6	-3.6	-3.7	-8.8	-5.7	7.2	4.7	5.1	7.2	9.0	-0.1	8.5	2.4	-2.8	1.3	2.6	0.6	-2.5	7.6	2.5	3.7	14.0	20.1	12.7	12.7	11.7
	3	-4.7	-5.5	-4.4	-1.5	-4.4	-3.9	5.9	-4.1	-1.0	-1.3	2.6	-0.3	-3.6	1.6	-2.3	1.9	1.9	1.4	-4.5	1.5	4.7	0.5	0.6	2.3	3.9	0.8	2.4	10.8
	4	2.6	2.5	3.8	5.8	7.9	3.6	2.9	-2.5	1.3	-2.7	-1.3	-2.6	-3.3	-0.8	5.9	5.3	6.6	10.5	8.4	7.2	7.9	-6.1	-2.9	-3.6	4.0	1.0	9.2	-4.3
	5																												
	6	3.9	7.6	6.0	9.8	4.8	7.9	6.7	0.6	-2.4	1.0	0.3	1.9	0.8	-1.4	2.7	7.5	4.3	7.6	3.0	6.4	3.4	-7.3	-2.9	-5.4	2.3	0.1	-4.3	-4.9
	7	2.2	-0.4	1.5	4.4	5.7	7.4	-2.6	0.2	-1.4	-0.8	1.9	7.2	2.4	-2.8	7.5	9.1	1.7	12.6	17.7	10.3	3.6	8.4	-0.3	4.3	1.0	12.2	6.5	-1.3
	8	-2.8	2.9	2.3	1.6	1.3	-3.5	-0.5	1.5	0.9	5.6	6.3	3.5	-1.3	0.0	-1.7	-0.1	2.2	4.0	4.6	-1.6	-2.5	17.8	14.1	15.6	12.1	8.9	6.4	12.6
	9	-3.2	-0.8	1.5	4.7	0.5	-2.3	-3.1	-0.8	0.1	1.2	4.9	2.2	-0.7	-0.7	4.0	6.5	8.4	14.1	14.2	7.3	3.3	6.9	2.0	3.9	14.7	17.8	12.7	3.3
	10	-8.3	-9.4	-8.0	-7.2	-3.5	-11.1	-10.8	0.7	-3.0	2.4	6.3	8.2	1.4	-0.5	9.5	8.6	16.1	16.6	15.3	11.4	9.3	6.6	4.6	5.9	13.2	13.9	9.9	4.0
	11	-3.6	-3.7	-1.7	-4.5	-3.9	-5.5	-6.1	-2.4	-2.8	4.5	-3.4	-0.3	-6.3	-6.0	4.1	1.7	10.1	3.1	4.2	1.7	0.8	-2.9	-0.8	-0.3	0.0	0.7	-0.4	-1.3
	12	-3.7	-3.7		-1.2	0.1	-4.8	0.4	0.9	-0.5		2.3	3.2	-0.8	-3.1	1.8	2.1		7.4	3.8	1.9	4.8	-4.5	-3.7		2.7	3.1	0.7	-0.1
	13	-3.5	-2.6	-0.9	3.2	1.5	-4.0	-7.9	11.4	9.1	14.5	14.5	14.5	10.2	10.6	3.0	-1.2	5.5	14.9	8.9	-4.8	-2.1	-3.7	-1.8	1.4	4.0	2.3	-3.9	-2.4
	14	-0.1	0.3	-0.4	-2.2	1.2	-3.2	-3.0	5.7	6.6	4.2	6.1	12.0	-1.2	2.6	8.7	5.5	3.9	7.8	11.0	7.6	4.7	9.4	9.9	6.1	10.3	18.6	2.4	9.8
	15	-9.7	-10.5	-4.7	-5.4	-8.5	-9.5	-10.4	-10.8	-8.8	-5.7	-6.9	-3.6	-10.1	-5.3	-7.0	-4.3	0.3	-3.2	-0.9	-4.8	-4.5	-1.5	-7.9	0.0	5.2	2.0	-0.1	-1.4
	16	1.6	-0.5	-1.3	-0.9	-1.2	-2.1	0.3	-1.0	-0.8	-1.7	3.1	0.1	3.5	-2.5	4.8	-4.0	-3.3	3.7	1.2	0.2	-3.9	-2.3	0.1	-1.2	7.3	4.7	4.3	-3.5
	17	1.0	1.1	2.5	3.5		3.0	0.2	5.7	4.0	5.1	7.8		7.1	6.1	-2.1	-1.5	1.6	2.7		2.7	-1.5	-4.9	0.7	-1.0	-1.4		0.7	-3.8
	18																												
	19	1.7	0.8	2.0	-1.9	2.5	-0.4	2.2	2.7	5.6	4.4	2.3	8.4	4.1	0.8	8.0	5.7	1.7	4.2	10.9	0.3	0.5	8.0	8.2	9.9	7.3	9.8	7.9	12.3

Ear		L	L	L	L	L	L	L
Frequency (kHz)		6	6	6	6	6	6	6
Session number		1	2	3	4	5	6	7
Subject number	1	-2.4	-1.6	-1.8	-3.8	-4.1	1.9	-2.4
	2	8.4	14.6	9.9	10.0	7.3	14.6	11.7
	3	5.5	9.2	8.8	9.9	17.8	6.8	0.1
	4	-7.9	-2.2	-3.9	0.8	-0.7	0.8	-5.8
	5	-1.7	-3.9	1.7	5.7			-0.3
	6	16.6	22.5	18.2	23.8	19.3	13.7	13.9
	7	-0.7	1.2	4.7	8.7	18.5	3.6	-1.2
	8	-1.7	9.0	8.2	7.2	7.7	4.1	3.5
	9	8.7	1.5	2.9	8.1	12.3	15.5	13.1
	10	19.6	15.1	17.7	21.0	29.5	20.5	20.0
	11	10.1	12.0	5.3	12.0	17.4	11.7	12.2
	12	-1.8			5.5	2.9	2.3	-1.4
	13	7.8	5.8	28.3	18.8	21.9	20.7	19.1
	14	11.2	10.2	10.3	10.2	7.1	8.5	8.7
	15	8.3	1.3	7.1	1.6	4.6	7.6	-4.4
	16	4.9	19.2	12.9	12.3	21.8	14.5	6.4
	17	26.5	13.6	19.6	13.6		20.0	7.8
	18							
	19	2.0	4.3	7.6	2.8	6.2	1.7	-0.7

Ear		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Frequency (kHz)		1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4	4	4
Session number		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Subject number	1	-1.2	-3.0	-0.4	4.9	0.9	-2.8	-5.2	3.5	1.3	3.4	6.9	7.2	4.4	0.4	1.0	-0.2	0.6	1.1	6.8	-0.5	-2.7	-3.3	-1.9	13.0	16.3	8.8	0.4	5.6
	2	-2.6	-4.3	-0.3	-2.4	-1.4		-3.2	7.2	9.8	8.0	8.5	9.6	5.4	8.5	4.5	4.2	8.2	5.3	7.1	6.3	6.7	9.3	4.9	6.6	14.0	10.0	15.2	2.7
	3	-1.3	-0.9	1.3	1.7	0.7	2.3	-2.5	6.3	4.5	1.9	4.9	4.4	7.3	-0.5	1.7	0.2	-1.7	3.0	6.3	0.2	2.8	-2.6	6.4	1.1	10.7	4.3	11.6	0.8
	4	0.1	-1.4	-0.9	1.7	3.3	0.9	-0.7	8.7	4.9	6.3	5.1	3.5	4.9	6.5	-1.6	2.5	2.5	7.7	8.7	7.7	2.6	-3.3	10.1	-0.9	7.2	4.3	5.8	-0.2
	5																												
	6	0.4	-0.9	-1.8	-3.6	-0.7	-1.6	-4.1	4.9	5.5	2.2	-2.4	8.0	5.1	6.8	4.7	3.2	-0.5	5.2	0.7	2.8	2.0	-0.1	-2.0	1.4	-6.4	-0.4	-2.3	-4.9
	7	0.8	-3.1	5.3	5.0	13.3	2.6	1.1	-1.9	6.3	-1.5	0.8	11.7	-2.9	-2.3	5.6	5.4	3.0	11.3	13.9	5.4	3.4	-2.0	5.8	-2.9	15.2	20.3	4.8	8.2
	8	1.1	0.6	0.7	1.1	-2.3	-2.6	-2.8	3.6	0.9	4.9	5.6	3.3	0.5	-1.1	9.5	10.9	17.9	13.2	9.9	9.7	7.1	10.8	5.5	4.6	8.2	14.7	4.0	3.0
	9	4.0	-2.3	2.5	0.4	4.1	7.1	1.2	-0.7	-2.1	0.3	-0.9	-0.2	0.8	-3.2	-3.6	-2.2	-1.6	0.4	1.7	-3.5	-3.3	-7.6	4.3	11.6	15.1	11.0	10.5	1.1
	10	-6.5	-3.6	-6.4	2.1	-5.0	-3.3	-9.0	1.1	3.3	3.2	10.5	8.5	5.3	-0.4	2.7	3.7	3.2	6.3	8.1	7.3	-0.3	-1.3	1.9	0.3	15.7	17.1	5.2	1.9
	11	-0.9	-4.3	-7.1	-2.8	-4.8	-2.4	-5.8	1.3	-1.6	-2.9	2.6	-3.1	-1.2	2.6	2.2	4.7	-1.9	8.3	1.3	-2.0	6.4	-0.4	-2.9	-1.7	1.8	0.2	3.9	-4.9
	12	-2.0	-1.9		-0.2	0.8	-1.7	0.7	1.4	-4.2		5.5	-0.5	-5.3	2.5	6.0	10.3		11.9	11.5	9.7	12.0	8.0	9.8		15.0	13.6	13.5	9.3
	13	-0.7	1.6	-0.2	8.3	-1.4	-0.7	-4.2	8.8	13.0	7.6	15.6	10.4	6.6	5.6	9.7	12.4	9.7	14.3	4.0	4.6	9.7	3.1	1.2	1.8	7.2	2.2	-1.3	0.8
	14	-2.3	-2.8	-4.0	-2.2	-2.6	-4.4	-4.0	0.0	0.0	-1.0	5.4	4.1	0.6	1.1	0.9	1.0	-0.1	8.1	4.1	2.9	0.1	-1.0	-1.9	-0.7	2.2	2.6	-2.1	-0.4
	15	-8.5	-7.9	-4.8	-5.1	-4.4	-7.1	-7.5	-9.3	-6.1	-4.8	-3.6	-3.2	-4.1	-6.1	-5.3	-6.5	-2.1	-1.1	8.8	-3.0	-5.1	0.7	-3.4	1.4	5.1	5.8	4.3	1.2
	16	-1.2	-2.3	-3.2	-2.2	-3.1	-3.8	-5.7	-2.5	-4.6	-6.3	-5.8	-4.4	-7.8	-6.6	-1.5	-2.6	-3.5	0.6	2.8	-3.7	-3.3	3.5	2.4	-1.2	1.4	7.1	-3.4	-0.4
	17	-4.2	3.4	0.9	4.6		1.4	-3.3	-4.8	1.2	1.9	2.6		1.3	-2.8	-6.9	-4.5	-1.6	-0.3		-1.6	-8.4	-8.8	-2.8	-3.0	1.8		0.7	-11.5
	18																												
	19	1.9	4.3	2.0	0.4	2.6	-1.7	2.8	6.8	4.0	13.0	9.1	12.2	8.0	6.3	6.9	4.0	7.6	9.5	7.3	2.5	3.7	10.2	11.4	18.7	12.5	24.2	17.4	15.5

Ear		R	R	R	R	R	R	R
Frequency (kHz)		6	6	6	6	6	6	6
Session number		1	2	3	4	5	6	7
Subject number	1	-2.6	-7.5	-3.3	-3.6	-1.3	0.9	-6.1
	2	10.7	18.3	16.7	10.0	19.9	7.3	21.5
	3	6.7	2.6	14.0	18.5	2.4	9.8	9.4
	4	-14.7	-6.7	-11.1	-3.0	-2.5	-4.4	-8.8
	5	16.9	28.4	16.5	12.2			18.3
	6	4.8	8.6	28.3	14.0	15.7	9.6	6.3
	7	-5.4	-2.8	8.5	6.8	13.0	2.9	1.1
	8	2.0	2.2	6.7	10.9	6.0	6.5	3.3
	9	1.5	12.0	0.8	7.6	4.4	2.0	7.0
	10	21.9	12.2	16.8	25.6	23.1	16.5	8.8
	11	13.9	9.1	13.3	15.7	11.5	12.8	10.4
	12	2.6	-2.5		2.2	9.6	-3.2	4.2
	13	9.3	15.0	20.3	5.8	21.2	8.7	11.5
	14	-6.7	-17.5	-20.3	-3.6	-11.5	-11.9	-7.2
	15		-6.8	1.2	2.0	3.5	1.8	4.4
	16	12.6	10.0	11.9	10.0	20.7	10.3	7.1
	17	10.6	2.2	0.0	-0.6		2.8	7.3
	18	19.9	18.2	21.8	25.8		24.2	19.3
	19	3.5	10.1	7.1	14.3	5.0	-0.7	2.9

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