Ph.D. thesis, September 1995 - September 1999

MECHANISMS OF AMINOGLYCOSIDE OTOTOXICITY IN HUMANS

S.A. Burr

Centre for Mechanisms of Human Toxicity

University of Leicester.

Principal Supervisor Dr. D.E. Ray

Neurotoxicology Section

Medical Research Council Toxicology Unit.

Associate Supervisor Prof. K.L. Woods

Department of Medicine and Therapeutics

Leicester Royal Infirmary.

Acting Supervisor Dr. M. Mulheran

Neurotoxicology Section

Medical Research Council Toxicology Unit.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ABSTRACT

MECHANISMS OF AMINOGLYCOSIDE OTOTOXICITY IN HUMANS

by S.A. Burr, MRC Toxicology Unit, University of Leicester, LE1 9HN.

Aminoglycoside ototoxicity is a common clinical problem. One patient group suitable for study are those with cystic fibrosis (CF); as recurring chest infections are repeatedly treated with intravenous administration of the aminoglycoside antibiotics gentamicin or tobramycin. Development of techniques that enable the sub-clinical detection of early changes in auditory function could provide a means of monitoring for ototoxic side-effects and facilitate more effective therapeutic management. Furthermore, by non-invasive measurements of deficits in different aspects of cochlear performance, the sites and progression of damage can be studied directly in the human.

The mechanistic approach adopted here has been to separate out differences in: auditory sensitivity (attributed to the whole auditory pathway and measured by pure tone audiometry); cochlear selectivity (attributed to inner and outer hair cells and measured by frequency resolution); and outer hair cell function (as measured by oto-acoustic emissions). The normal inter- and intra-subject range and test re-test variability was assessed for each method of measurement using approximately sex- and age-matched non-CF subjects. The primary end-points for all audiometric tests being the: effect of age in control subjects; dose-response relationship in patients; and direct comparison of control and patient groups.

106 control subjects and 78 patients were tested. Over the range of ages investigated (from 10-37 years for most tests) there was only a correlation with age ($p < 0.05$) for high frequency pure tone thresholds at 10, 12, 14 and 16 kHz, and mean differences in frequency resolution at 4 and 8 kHz. Therefore, for comparison of control subjects and patients it was only necessary to subdivide into juvenile and adult groups with those tests. There were numerous factors confounding the establishment of any dose-response relationship. In particular, there were found to be almost insurmountable problems associated with the retrospective calculation of patient aminoglycoside exposure. Nevertheless, 7.7 % of patients (control adjusted) did have high frequency sensorineural hearing loss that coincided in onset frequency with deficits in distortion-product oto-acoustic emissions. Also, aminoglycoside exposed patients as a group had significantly more saturating distortion-product input-output functions ($p < 0.05$). Surprisingly no more subtle changes were apparent. However, these physiological findings are still consistent with progressive damage affecting outer before inner hair cells. It would appear that most CF patients may be protected in some way, unless predisposed to ototoxicity by some additional risk factor(s). An additional much larger prospective study examining the efficacy and safety of aminoglycosides in CF patients will extend these findings and be taken forward by others.

ACKNOWLEDGEMENTS

I thank Dr. D.E. Ray (Head of Neurotoxicology, M.R.C. Leicester) for his honest and friendly guidance. I am consequently indebted to Dr. M. Mulheran (post-doctoral scientist, Dr. Ray's research group), for his enthusiasm towards clinical collaboration and the invaluable work he did in building the foundations that made this project possible. I am grateful to Prof. K.L. Woods (Consultant Physician, Leicester Royal Infirmary) for his helpful disposition and constructive criticism. I am also grateful to his research assistant Mrs. S. Fletcher, for her initial role in the administrative organisation of subjects. Without the participation of many control volunteers (from Leicester Grammar School and Leicester University) and patients (from Leicester Royal Infirmary, Birmingham Heartlands Hospital, Northampton District Hospital and Peterborough District Hospital) this project would not have been possible. My thanks to Dr. C. O'Callaghan, Dr. D.A. Woolf, Dr. D.E. Stableforth, Mr. D.W. Morgan, Dr. N.K. Griffin and especially Dr. J. Collinson for their clinical co-operation. Specialist knowledge was sought and obtained from: Prof. D.T. Kemp (Institute of Laryngology and Otology, University College London) on oto-acoustic emissions; Dr. R.A. Swann (Medical Microbiology, Leicester Royal Infirmary) on aminoglycoside dosage; and, Dr. M. Festing (M.R.C. Toxicology Unit, Leicester University) on statistical analysis. I would particularly like to thank Dr. C. Degg (Senior Medical Physicist, Leicester Royal Infirmary) who contributed indispensable advice and technical support throughout. This three year project was funded by a Medical Research Council studentship (reference G78/4526). Finally, most of all I thank Dr. Yee-Ling Leung for always being there when I needed someone.

The author claims no conflict of interest and has sole responsibility for any errors contained herein.

ABBREVIATIONS

Throughout the text the following abbreviations are used:

ORGANISATIONS

B&K	Bruel and Kjaer
BSI	British Standards Institute
IEC	International Electrotechnical Commission
ILO	Institute of Laryngology and Otology
ISO	International Organisation for Standardisation
MRC	Medical Research Council
NPL	National Physics Laboratory

UNITS OF MEASUREMENT

dB	Decibels
daPa	Decapascals
HL	Hearing Level
Hz	Hertz
RETSPL	Reference Equivalent Threshold Sound Pressure Level
SPL	Sound Pressure Level

OTHER RECOGNISED ACRONYMS

ATP	Adenosine Tri-Phosphate
CD	Compact Disc
CF	Cystic Fibrosis
CSSOAE	Click-Synchronised Spontaneous Oto-Acoustic Emission
df	Degrees of freedom
DPOAE	Distortion Product Oto-Acoustic Emission
IHC	Inner Hair Cell
i.m.	Intramuscular
i.v.	Intravenous
n	Number in sample
OAE	Oto-Acoustic Emission
OHC	Outer Hair Cell
p	Probability
PTA	Pure Tone Audiometry
PTS	Permanent Threshold Shift
r	Correlation coefficient
r^2	Coefficient of determination
SD	Standard Deviation
SOAE	Spontaneous Oto-Acoustic Emission
TTS	Temporary Threshold Shift

CONTENTS

INTRODUCTION

1.1. BACKGROUND

1.1.1. RECENT ADVANCES

Over the past few decades there has been a rapid expansion of the understanding of the peripheral auditory system. This has arisen because of the application of established and novel techniques that enable the separation of different functional sites. The main advances that have contributed to a much stronger understanding of how the physiological properties of the cochlea are determined by its underlying structure include:

- Identification of a 'tonotopic map' in the cochlea (reflected in cochlear nerve fibre activity);
- Identification of the main energy source which enables active processing by the cochlea (endocochlear potential);
- Understanding of the separate physiological roles played by the two distinct groups of sensory cells in the cochlea (inner and outer hair cells);
- Discovery of oto-acoustic emissions which result from active processes in the cochlea, hence reflecting the functional status of one group of sensory cells (electromechanical activity of outer hair cells);
- Validation of psychophysical techniques that reflect cochlear functioning; and,
- How the nerve fibre activity reflects an active filtering process that is dependent on the integrity of all structures in the cochlea.

In combination, all of the above advances now position us to investigate the sites and mechanisms of cochleotoxic agents in humans at therapeutically relevant doses.

1.1.2. PRESENT AND FUTURE STUDY

Novel psychophysical and physiological techniques can be used to elucidate the mechanism of the early stages of cochleotoxicity in humans. Different aspects of cochlear performance can now be non-invasively and repeatedly assessed in well-defined patient groups. One group at risk from aminoglycoside ototoxicity is patients with cystic fibrosis, who are likely to receive long term antibiotic therapy with gentamicin and tobramycin. The lack of consensus on the dose-response relationship of aminoglycoside ototoxicity needs to be resolved. In particular, animal models are based on supra-toxic doses and require confirmation by comparison with the actual therapeutic regimes used in humans. The benefits of the research are two-fold: increasing the scientific understanding of ototoxic processes and optimising drug dosage in clinical management.

1.2. AIMS

The rationale behind this study was to non-invasively investigate the progression of deficits in different aspects of cochlear performance associated with gentamicin and tobramycin therapy in cystic fibrosis patients. In particular, the hypothesis was tested that it is possible to measure sub-clinical changes in OAEs, and /or frequency resolution, before PTA (for specific objectives see methods 2.6.1.). Such changes would be consistent with outer hair cells being affected before inner hair cells, thus confirming the animal model of action directly in humans.

1.2.1. CLINICAL IMPLICATIONS

Monitoring for the side-effects of potentially ototoxic therapeutic agents can be achieved by extensive audiological testing coincident with drug-serum assays. The dosage of drug administered to patients can thus be optimised by making informed decisions. Over-dosing can be

prevented by decreasing the dose sufficiently to minimise or eliminate ototoxic side-effects, and under-dosing can be prevented by enabling a safe increase in dose to improve treatment of the existing condition. Iatrogenic hearing loss can therefore be kept to a minimum.

If deafness progresses unchecked, a patient's quality of life can suffer severely. Furthermore, considerable economic cost is associated with the subsequent diagnosis, treatment, rehabilitation, education and medical-legal cases of patients suffering from drug-induced hearing loss. Therefore, the increased use and consequent validation of more selective, non-invasive hearing tests, will be of value to establish safer therapeutic regimes and for the earlier detection of many types of auditory dysfunction in humans.

1.2.2. SCIENTIFIC IMPORTANCE

Investigation of ototoxic mechanisms is crucial to the development and application of safe pharmacological and chemical agents in both therapeutic and industrial settings. In particular, any insights into ototoxic mechanisms gained by human research may enable the revision of inappropriate animal models. Furthermore, an understanding of the aetiology of ototoxicity is of considerable use in establishing how the elements of the auditory pathway inter-relate in response to sensory input. One possible approach is to separate the deficits in cochlear function into measurements of sensitivity, frequency selectivity and outer hair cell activity. However, the basic structure, function and diseases of the cochlea must first be understood and interrelated in order to be able to map the targets and progression of damage that occurs with aminoglycoside ototoxicity.

1.3. COCHLEAR ANATOMY

1.3.1. COMPONENTS OF THE INNER EAR

The cochlea is the conical part of the inner ear that is found almost horizontally anterior to the vestibule and forms the anterior part of the labyrinth. In man, the average cochlea is 10 mm in diameter, 5 mm in height from base to apex, and consists of 2.5 coils which total 35 mm in length. The cochlea is compartmentalised into a series of ducts by a diverse range of membranes (Fig. 1.3.). All of the cochlear components are collectively concerned with hearing. Central to the structural organisation of the cochlea is the organ of Corti, containing the sensory hair cells that are responsible for converting auditory stimuli into neural signals. (For a comprehensive anatomical review see Berry et al., 1995.)

1.3.2. DUCTS OF THE COCHLEA

The scala media (endolymphatic duct, cochlear duct, or ductus cochlearis) consists of a spiral tube containing endolymph that is found lying on the outer wall in the bony canal (spiral lamina) of the cochlea, between the scala vestibuli and scala tympani. The scala vestibuli and scala tympani contain perilymph and are joined by a single opening called the helicotrema at the apical end of the cochlea. The scala vestibuli is the upper tube of the cochlea that extends from the oval window (fenestra ovalis) at the basal end of the cochlea, to the helicotrema. Conversely, the scala tympani is the lower tube of the cochlea that extends from the helicotrema to the round window (fenestra rotunda) at the basal end of the cochlea. The cochlear aqueduct (perilymphatic duct) is a channel containing perilymph passing through the temporal bone that connects the scala tympani of the cochlea to the subarachnoid space.

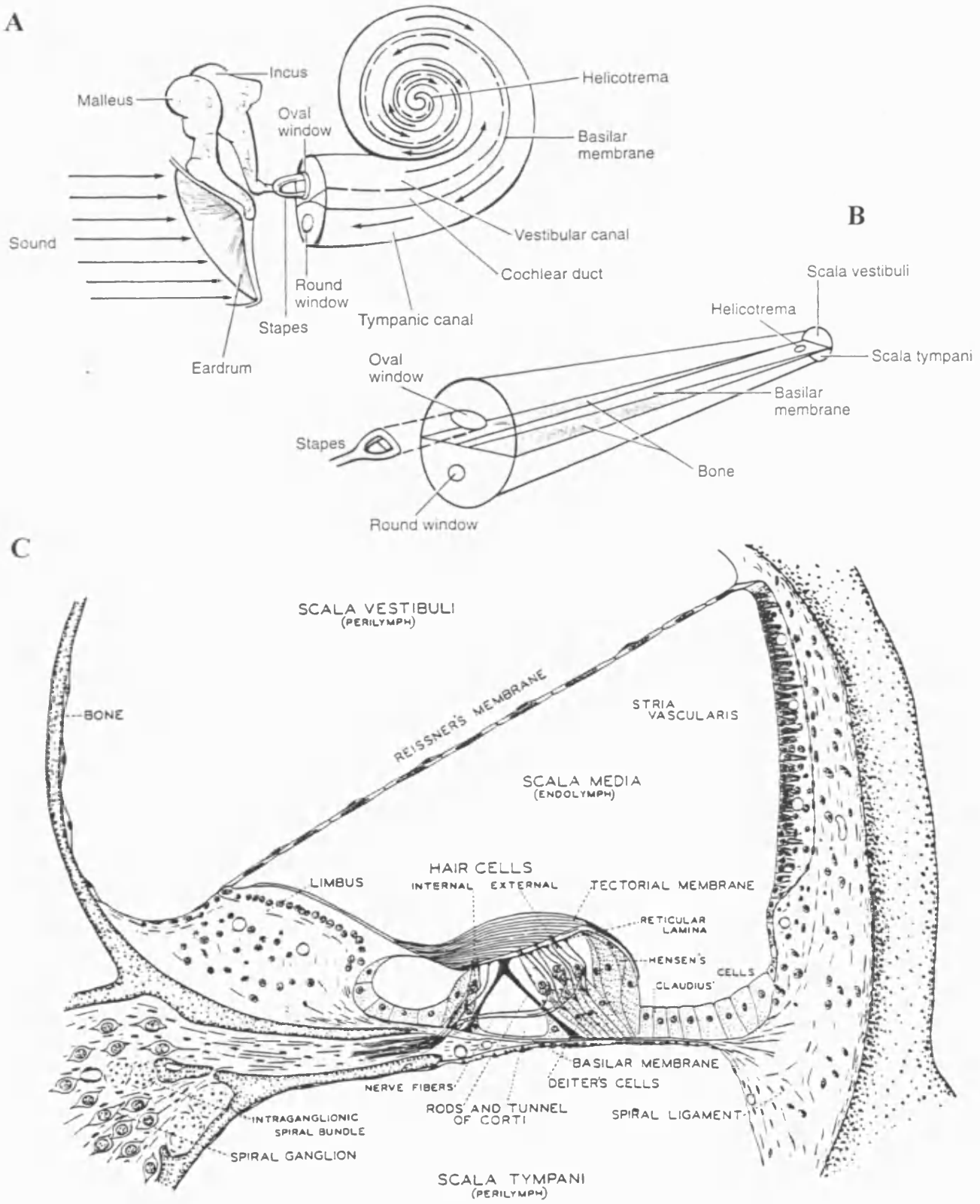


FIG. 1.3. Structural features of the cochlea. (**A**) Conductive pathway of sound to the coiled cochlea, via the middle ear coupled via the oval and round windows (adapted from Yin, 1995). (**B**) Change in cochlea dimensions from base to apex, illustrated by showing the cochlea uncoiled (adapted from Yin, 1995). (**C**) Organ of Corti and associated hair cells, shown in transverse section (adapted from Davis et al., 1953).

1.3.3. MEMBRANES OF THE COCHLEA

Reissner's membrane constitutes two cell layers of 10-15 μm total thickness, separating the scala vestibuli from the scala media. The stria vascularis is a highly vascularised epithelium composed of three layers of pigmented granular cells and located on the outer wall of the scala media. The spiral lamina is a bony plate that extends outwards from the modiolus and forms part of the structures that divide the cochlea into sections. The spiral limbus is a vascularised connective tissue that arises from the spiral lamina and supports the tectorial membrane. The tectorial membrane is an acellular fibrous structure that overlies the basilar membrane. The basilar membrane stretches from the spiral lamina to the basilar crest, with the inner part supporting the organ of Corti. The basilar membrane increases in width from 150 μm at the base to 250 μm at the apex, and decreases in thickness from 7.5 μm at the base to 1.4 μm at the apex (Spoendlin, 1972; Fig. 1.3.B.).

1.3.4. ORGAN OF CORTI

The organ of Corti (Corti's organ, or spiral organ) named after Alfonso Corti (1822-1888), contains the sensory cells for hearing and is composed of a series of epithelial structures situated on the inner part of the basilar membrane (Davis et al., 1953; Fig. 1.3.C.). The labyrinth supporting cells form the framework for the optimal orientation of sensory cells within the organ of Corti. The tunnel of Corti is an endolymph filled space between the basilar and tectorial membranes. The spiral ganglion (ganglion of Corti) is the sensory ganglion of the cochlear nerve, the cells of which send fibres centrally to the cochlear nuclei of the brain stem and peripherally to the cochlear hair cells.

1.3.5. HAIR CELLS

In humans, the organ of Corti typically contains 15,000-25,000 hair cells (Ulehova et al., 1987). Fundamentally there are two distinct types of hair cell: inner hair cells (IHCs) and outer hair cells (OHCs) (Spoendlin, 1984; Fig. 1.3.C.). Each hair cell arises from the basilar membrane and is tipped by 50-100 cross-linked hair-like projections (microvilli) called stereocilia. The tips of the tallest stereocilia on the OHCs are imbedded in the tectorial membrane, whereas those of the IHCs are not. IHCs are 'bulbous' shaped cells, 35 μm long and 10 μm at their widest diameter. IHCs are arranged in a single medial row along the spiral of the organ of Corti. There are typically 3,000-5,000 IHCs each with stereocilia arranged in 3-5 U-shaped rows. OHCs are 'angular' shaped cells, 8-10 μm in diameter with a length increasing from 20-30 μm at the base to 80-100 μm at the apex of the cochlea. OHCs are arranged in rows of between three and five, and located further from the modiolus than the single row of IHCs. There are typically 12,000-20,000 OHCs each with stereocilia arranged in 3 V or W-shaped rows. Therefore, there are 3,000-5,000 hair cell 'units' (1 IHC with 3-5 OHCs) in series and evenly spaced along the spiral of the organ of Corti. (For a review see Pickles, 1988.)

1.3.6. COCHLEAR NERVE

Most cochlear nerve (auditory nerve, or non-vestibular portion of VIIIth cranial nerve) fibres pass either as afferent connections to the cochlear nuclei or as efferent connections from the superior olivary complex and the olivocochlear bundle. The IHCs receive the majority of their efferent connections ipsilaterally from the lateral system, whereas the OHCs receive the majority of their efferent connections contralaterally from the medial system. There are approximately 28,000 afferent connections and 1800 efferent connections with the hair cells of the organ of Corti. Each IHC typically has up to 10 afferent connections, whilst each OHC typically has 1-3 afferent connections (Spoendlin,

1970). The afferent connections of IHCs are individual parallel connections, whereas the afferent connections of the OHCs are shared by a series of synapses with up to ten other OHCs in a basal direction from the spiral ganglion (Spoendlin, 1978). In total, IHCs indirectly receive 1,000 efferent connections that terminate on the dendrites of their afferent connections. In contrast, OHCs directly receive approximately 800 efferent connections. Each OHC typically receives 6-10 efferent terminals near the basal end, which decrease in number towards the apical end of the organ of Corti (Spoendlin, 1984). Overall, the pattern of cochlear innervation reveals 90-95 % of all connections are afferent and 90-95 % of all afferent connections are direct from IHCs. It is the finding of these differences in innervation between inner and outer hair cells that leads to the realisation that there must be corresponding differences in function. For example, obviously OHCs can have comparatively little involvement in transmitting information to the brain. All of the structural differences and interrelationships between the two hair cell types are then of fundamental importance in gaining insight into the active processing involved in cochlear physiology, as we shall see next.

1.4. COCHLEAR PHYSIOLOGY

1.4.1. THE OUTER AND MIDDLE EARS

The outer ear consists of the visible 'ear' (pinna) and ear canal (external auditory meatus), which channel sound from the environment to the eardrum (tympanic membrane) of the middle ear. The middle ear then acts as an acoustic impedance transformer to efficiently conduct sound from air in the environment to liquid in the cochlea. Energy transduction is facilitated by a buckling motion of the tympanic membrane; lever action of the three ossicles (malleus, incus and stapes bones) in the middle ear; and reduction in surface area from the tympanic membrane to the fenestra ovalis of the cochlea (Fig. 1.3.A.).

1.4.2. THE INNER EAR - A HISTORICAL PERSPECTIVE

There have been several milestones in our understanding of the nature of the inner ear. Perhaps the most significant contribution this century was the identification of a 'tonotopic map' in the cochlea (Von-Bekesey, 1947; reflected in cochlear nerve fibre activity; Tasaki, 1954). This was closely followed by the identification of the main energy source which enables active processing by the cochlea (the endocochlear potential; Tasaki & Spyropoulos, 1959). It was then realised that cochlear nerve fibres exhibited 'sharp tuning' (Evans, 1972) when compared with the broad tuning of the basilar membrane observed by Von-Bekesey, and to explain this discrepancy a 'second filter' was required (Evans & Wilson, 1975). However, the tuning of the basilar membrane and cochlear nerve fibres was later found to be similarly sharp (Rhode, 1978; and, Johnstone et al., 1986). At about this time, oto-acoustic emissions - resulting from active processes in the cochlea - were discovered (Kemp, 1978; see introduction 1.8.7-10.). The two distinct groups of sensory cells in the cochlea (inner and outer hair cells) were then realised to have separate physiological roles. Specifically, oto-acoustic emissions were suspected of reflecting the functional status of

outer hair cells, and these cells were then found to be electromechanically active (Brownell et al., 1985; and Ashmore, 1987). At a comparable time, psychophysical techniques that reflect cochlear functioning - and in particular tuning - were being comprehensively validated, principally by Moore (e.g. Moore & Glasberg, 1983; see introduction 1.8.5-6.). Finally, how the nerve fibre activity reflects an active filtering process that is dependent on the integrity of all structures in the cochlea - particularly the dependence of 'sharp tuning' on outer hair cell function - has been demonstrated (Liberman & Dodds, 1984; Neely & Kim, 1986).

In the light of these findings, there are some key features of cochlear function that can now be considered separately and in more detail. Namely: basilar membrane movement and the travelling wave; stereocilia deflection and opening of apical channels; endocochlear potential and transduction of signals; and, inner hair cells, outer hair cells and 'sharp tuning'.

1.4.3. BASILAR MEMBRANE MOVEMENT AND THE TRAVELLING WAVE

Cochlear mechanics process incoming sound in the form of pressure waves, separating differences in intensity and frequency (Von-Bekesey, 1947). The specific frequencies are identified by their absolute position of resonance along the organ of Corti, and are reflected in the subsequent tonotopic neural organisation (Tasaki, 1954). Thus, the cochlea appears to act as a Fourier analyser, whereby the different frequencies present in complex sounds are separated into the component sine waves.

Each pressure change incident at the oval window is effectively instantaneously equalised by a corresponding movement of the round window. The rapid vertical transition of energy across the basilar membrane at the basal end of the cochlea, generates a slower horizontal component that progresses along the scala vestibuli towards the helicotrema as a travelling wave (Von-Bekesey, 1947; Fig. 1.4.3.A.).

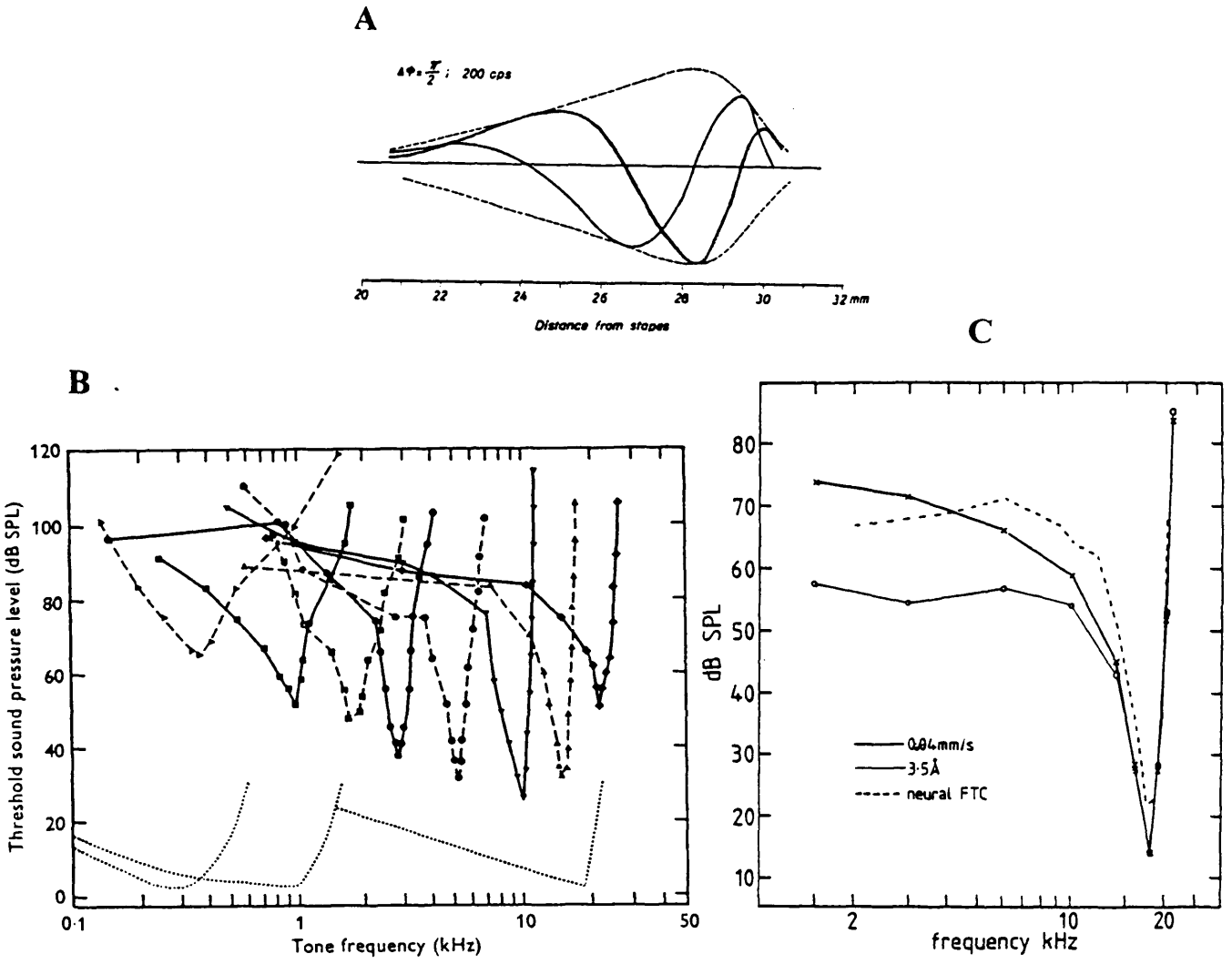


FIG. 1.4.3. Functional features of the cochlea. II. Transmission from travelling wave to tuning curve. (**A**) The longitudinal bending of the basilar membrane, measured in response to a 200 Hz tone. The position is shown: at one instant, and then one quarter of a cycle later by the full lines; and the envelope of movement by the outer broken curves. (Adapted from Von-Bekesey, 1947.) (**B**) Sharp tuning and characteristic frequencies of different positions along the guinea pig cochlea, showing variation in threshold with sound frequency. The upper set of tuning curves is for individual afferent cochlea nerve fibres. The lower set of dotted tuning curves is for the basilar membrane when the cochlea was in poor condition. (Adapted from Evans, 1972.) (**C**) Sharp tuning at one position on the basilar membrane in the guinea pig cochlea. The continuous lines show the sound intensities required to produce standard movements (a velocity of 0.04 $mm.s^{-1}$, or a displacement of 3.5 Angstrom units) of the basilar membrane at various frequencies. The dotted line shows a similar frequency-threshold curve for an individual nerve fibre with a similar characteristic frequency. (Adapted from Sellick et al., 1982.)

The point along the organ of Corti at which any frequency component of a sound will resonate, depends on the resistance of the fluid and membranes of the cochlea to movement. Progressing from base to apex: the cochlear ducts decrease in effective diameter, whilst the basilar membrane decreases in thickness and increases in width (drawn by Von-Bekesey, 1947; but not discussed until Spoendlin, 1972). Thus the travelling waves have a steeper apical side than basal side. Even with constant mass, stiffness and damping properties of the fluid with respect to the membranes, the dimensional changes, which manifest gradually from base to apex, are sufficient to enable differential resonance of decreasing frequencies at sequential points along the organ of Corti (Neely & Kim, 1986).

However, passive mechanical processing alone is insufficient to account for the sharp tuning of the sensory cells in the organ of Corti; as revealed by cochlear nerve fibre recordings (Evans, 1972) and intracellular microelectrode recordings from inner hair cells (Russell & Sellick, 1978). A 'second filter' was proposed by Evans and Wilson (1975) to account for the discrepancy in tuning between the basilar membrane and nerve fibres. However, Von-Bekesey's work showing a broadly tuned basilar membrane was undertaken using cochleae from cadavers and using very high stimulus intensities. Furthermore the discovery of a large energy supply within the cochlea in the form of the endocochlear potential (see introduction 1.4.5.), led to the realisation that the cochlea was physiologically vulnerable (Tasaki & Spyropoulos, 1959; Kuijpers & Bonting, 1969; Melichar & Syka, 1987; Offner et al., 1987). Thus, it seemed possible that the broad tuning observed by Von-Bekesey was due to the poor condition of preparations that were available at that time. Later, more technically advanced measurements confirmed that the basilar membrane and cochlear nerve were similarly sharply tuned (Rhode, 1978; Sellick et al., 1982 and, Johnstone et al., 1986). Therefore, there must be an active input of energy into the mechanical system in order to facilitate the enhanced contrast necessary

to distinguish between sound stimuli that are similar in intensity and frequency (first proposed by Gold, 1948; for a review see Dallos, 1992).

1.4.4. STEREOCILIA DEFLECTION AND OPENING OF APICAL CHANNELS

The hair cells located in the organ of Corti are mechanoreceptors that are sensitive to auditory stimuli. The hair cell accessory structures are arranged so that appropriate stimuli cause movement of the stereocilia at the tips of hair cells (Pickles, 1987). Each stereocilium has 1-4 ion channels of 0.7 nm diameter at its apex (Corey & Hudspeth, 1979b). The stereocilia apical channels are held neither fully open nor fully closed, but are in constant movement between the two states. Thus, the apical channels have no absolute threshold for being open or closed but have a relative probability, determined by mechanical input (Hudspeth, 1985). The process of opening and closing is relatively rapid, with a temperature dependent latency of 40 μs (at 22 °C *in vitro* for the bullfrog; Corey & Hudspeth, 1979a). At any point along the organ of Corti, when a pressure wave crosses from the scala vestibuli to the scala tympani, a difference in resonance occurs between the tectorial membrane and basilar membrane resulting in a shearing effect on the stereocilia at that point (Davis, 1958). Movement of the basilar and tectorial membranes up towards the scala media causes stereocilial deflection in the direction of the tallest stereocilia, stretching the 'tip-links' between stereocilia and increasing the proportion of open channels. Movement of the basilar and tectorial membranes downwards away from the scala media causes stereocilial deflection in the direction of the shortest stereocilia, compressing the 'tip-links' between stereocilia and increasing the proportion of closed channels (Davis, 1958; Pickles et al., 1984; Hudspeth, 1985; Fig. 1.4.4.). Thus, the mechanical input is thought to determine the relative probability of ion channels being open or closed.

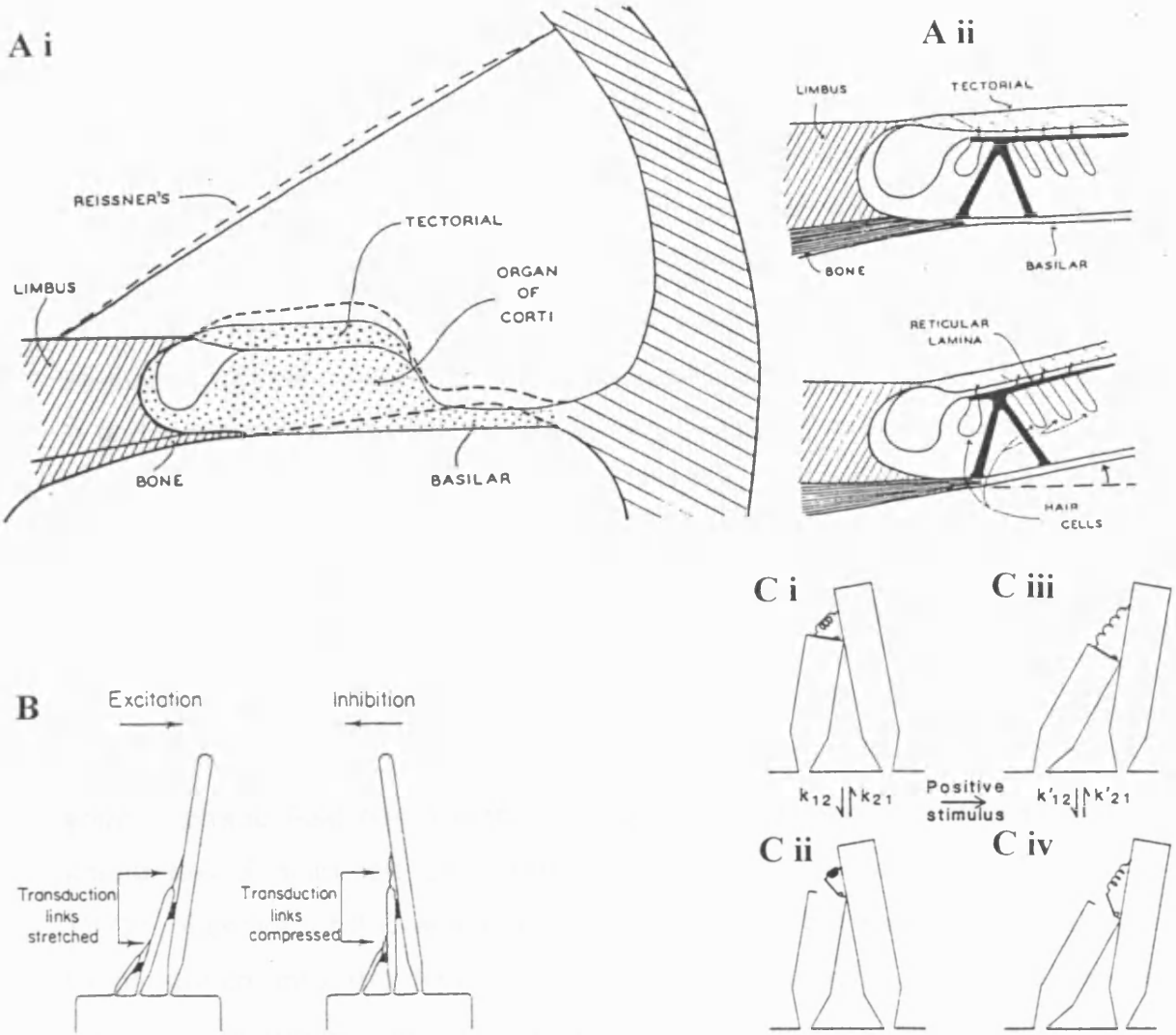


FIG. 1.4.4. Functional features of the cochlea. **I.** Transduction mediated by the shearing effect of basilar membrane movement on hair cell stereocilia. (**A i**) Probable pattern of displacement of structures in the cochlea, shown in transverse section (adapted from Davis, 1958). (**A ii**) Bending of the hair cell stereocilia caused by the shearing action of the tectorial membrane with respect to the reticular lamina (adapted from Davis, 1958). (**B**) Schematic sections through two hair cells, to show the links between stereocilia and the probable effect of movement in either direction due to shearing (adapted from Pickles et al., 1984). (**C**) A model for mechanicoelectrical transduction. At any instant each transduction channel at the tip of a stereocilium may be closed (**i**) or open (**ii**). The rate constants of opening and closing are k_{12} and k_{21} respectively, so the fraction of open channels is $k_{12} / (k_{12} + k_{21})$. When the stereocilium is deflected by an excitatory stimulus (**iii** and **iv**), the rate constant for opening increases and that for closing decreases, so the fraction of open channels increases and the hair cell becomes depolarized. Deflection in the opposite direction has the opposite effect, so the hair cell becomes hyperpolarized. (Adapted from Hudspeth, 1985.)

1.4.5. ENDOCOCHLEAR POTENTIAL AND TRANSDUCTION OF SIGNALS

The stereocilia are surrounded by the endolymph of the scala media. The endolymph is maintained at +80 mV with respect to the perilymph of the scala vestibuli and scala tympani (Von-Bekesey, 1952), by energy provided through Na^+/K^+ ATPase ion pumps in the stria vascularis. Experimental manipulations have also shown that a decrease in endocochlear potential coincides with a functional decrease in auditory sensitivity and selectivity (Tasaki & Spyropoulos, 1959; Kuijpers & Bonting, 1969; Melichar & Syka, 1987; Offner et al., 1987). Therefore, there is a large potential gradient across the stereocilia, which is essential for the normal functioning of the cochlea.

Perilymphatic fluid has a high sodium ion concentration of 140 mM and a low potassium ion concentration of 5 mM. Conversely, endolymphatic fluid has a high potassium ion concentration of 150 mM and a low sodium ion concentration of 2 mM (Johnstone & Sellick, 1972). Opening of IHC apical membrane channels allows potassium ions to be driven into the hair cell. Resulting depolarisation of the IHC facilitates neurotransmitter (glutamate) release, via voltage-gated calcium ion channels at the base of the cell. If sufficient glutamate diffuses across the synapse to the adjacent afferent axons a nerve impulse is activated, which is transmitted along the cochlear nerve towards the cochlear nucleus (Tasaki, 1954). Thus, movement of ions across IHCs is responsible for the transduction of a mechanical stimulus into a neural signal (reviewed by Hudspeth, 1985).

1.4.6. INNER HAIR CELLS, OUTER HAIR CELLS AND 'SHARP TUNING'

The IHCs appear to generate a response modulated by the degree of their mechanical stimulation, which is otherwise independent of their location within the cochlea (Cody & Russell, 1987). In other words, the characteristic frequency corresponding to a particular IHC is determined by the position of that hair cell along the organ of Corti.

In contrast to IHCs, OHCs have electromotile properties and act primarily as effectors in the cochlea (Brownell et al., 1985). Depolarisation causes contraction of the OHC body - by 20 nm for each mV change in potential - up to a change of 4 % in total length (Ashmore, 1987). In turn, OHC contractions lead to changes in the resonance characteristics of the tectorial membrane with respect to the basilar membrane. Even if cochlear dimensions were constant from base to apex, the change in mass, stiffness and damping properties of the membranes with respect to the fluid, caused by OHC activity, is sufficient to enable a compressed scale for resonance of different frequencies at sequential points along the organ of Corti (Neely & Kim, 1986). Therefore, a change in the degree of OHC contraction in a hair cell 'unit' (see introduction 1.3.5.) modulates the amount of mechanical stimulation incident at the IHC of that 'unit'. Thus, OHC activity is thought to augment the motions of the travelling wave (Ashmore, 1987).

OHCs have both fast and slow motile responses that can be considered as separate processes. The fast motile response depends directly on changes in current (Brownell et al., 1985), whereas the slow motile response depends on ATP and calcium ions and involves the formation of F-actin (Zenner et al., 1985). The fast motile response of OHCs acts to amplify signal transduction at the characteristic frequency whilst simultaneously damping immediately apically to the characteristic frequency. In contrast, the slow motile response of OHCs is facilitated by efferent stimulation, and the rate of contraction is relatively slow at 3-24 nm/sec (Zenner, 1986). The slow motile process acts in an adaptive capacity, varying the overall resistance of the organ of Corti to

resonance, thus enabling an increase or decrease in the degree of sensitivity of the IHCs to stimulation. Efferent neural connections from the olivocochlear bundle may therefore enable alteration of the level of 'gain' in the system (Mountain, 1980).

Thus, the precise geometrical relationships and physical characteristics of all the structures are extremely important in determining the function of the cochlea as a sharply tuned frequency analyser. Physically, the mass, stiffness and damping characteristics vary logorithmically from base to apex, and damage to the functional integrity of any element within the cochlea could lead to both an increase in threshold and a loss of sharp tuning.

1.5. COCHLEAR PATHOLOGY

1.5.1. SOURCES OF HEARING LOSS AND CONTRIBUTING FACTORS

All other sources of hearing loss must first be excluded before attributing any changes observed to a cochleotoxic origin. The main causes and effects of auditory damage are shown in Table 1.5. (for a general clinical guide see O'Donoghue et al., 1992).

1.5.2. COCHLEAR DAMAGE AND LOSS OF FUNCTION

Disturbance of normal cochlear function that exceeds the capacity for adaptation causes damage (for a review of the perceptual consequences see Moore, 1995). Deficits in different aspects of cochlear performance can be correlated with damage to specific structures at specific sites (Liberman & Dodds, 1984; Fig. 1.5.). Hence, damage to all cochlear elements would cause decreased sensitivity due to elevated thresholds; damage to both IHCs and OHCs would cause decreased selectivity due to broadening of the cochlear filters; and, damage to OHCs would cause decreased 'gain' due to decreased electromechanical amplification. Furthermore, damage to frequency-specific locations along the cochlea would cause a restricted frequency range due to a curtailed tonotopic map. Identification of the progression of structural damage in the cochlea should now be possible by the systematic separation of functional changes in sensitivity, selectivity and OHC activity, over a range of frequencies.

1.5.3. CLINICAL OTOTOXICITY

There are many potential targets for cochleotoxic drugs. The vascular supply to the cochlea may be compromised. The stria vascularis may be damaged, impairing exchange with the endolymph and disrupting the balance of the endocochlear potential. Also, the supporting cells of the organ of Corti, outer hair cells, inner hair cells and first order neurons can all be damaged separately or in combination. The progression of

damage due to cochleotoxins is thought to be from base to apex, whilst simultaneously progressing from OHCs to IHCs, before affecting the stria vascularis and finally the cochlear nerve (Quick, 1980). Many drugs are known to be cochleotoxic (for a general review see Harpur, 1982*), for example: quinine; salicylates such as aspirin; loop diuretics such as frusemide; anti-tumour agents such as cis-platin; and aminoglycoside antibiotics such as gentamicin and tobramycin.

* The review paper by Harpur (1982) was partly plagiarised and published in the same journal by Griffin (1988).

DAMAGE	OUTER EAR	MIDDLE EAR	INNER EAR	CENTRAL CONNECTIONS
CAUSES	• Infection • Cerumen • Trauma • Tumours	• Infection e.g. otitis media, cholesteatoma • Tympanic perforation • Ossicular dislocation • Otosclerosis • Eustachian tube dysfunction	• Ageing i.e. presbyacusis • Trauma • Infection e.g. meningitis, labyrinthitis • Congenital e.g. maternal rubella • Imbalance in nutritional supply e.g. hypoxia • Genetic susceptibility • Meniere's disease (idiopathic) • Radiation • Noise • Chemicals i.e. ototoxicity	• Tumours e.g. acoustic neuromas, meningiomas, dermoid cysts, cerebello-pontine angle tumours, astrocytomas • Brain stem diseases e.g. multiple sclerosis, syringobulbia, *herpes zoster varicella* • Cortical deafness
EFFECTS	Conductive hearing loss and symptoms of infection such as pain and discharge		Sensory hearing loss, tinnitus and / or disequilibrium	Neural hearing loss, tinnitus and / or disequilibrium

TABLE 1.5. Auditory pathology is classically divided into four main areas that can be affected: the outer ear; the middle ear; the inner ear; and the central connections.

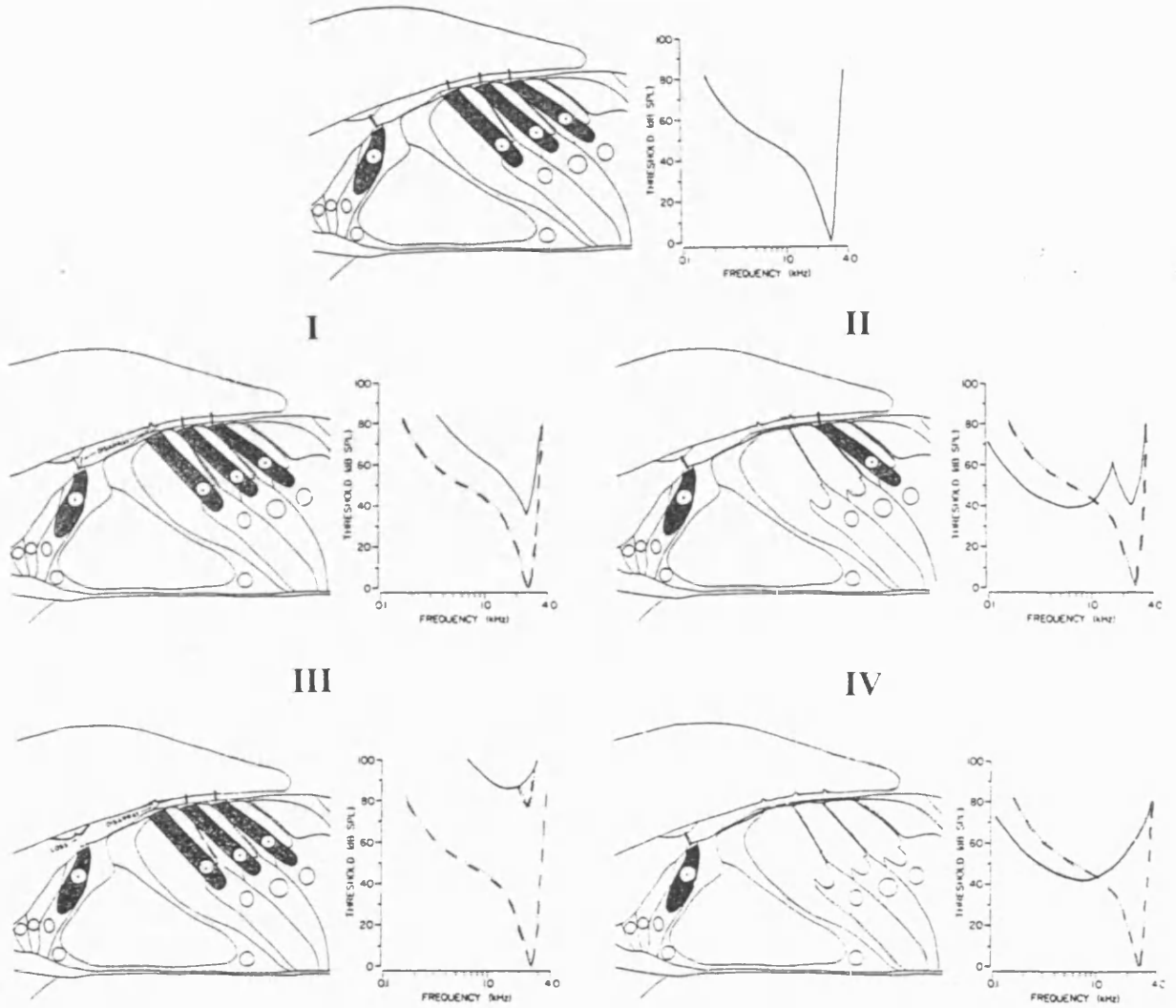


FIG. 1.5. Correlation between damaged structures and changes in functional performance of the cochlea. Semi-schematic representation of a normal organ of Corti (top) and four different damaged states (I-IV). Each damaged structural state is shown with the corresponding abnormal tuning curve from cochlear nerve fibres innervating such a region (normal tuning curve is shown as an overlying dashed line for comparison). (**I**) Poor coupling of stereocilia from IHCs and first row of OHCs to tectorial membrane. (**II**) Loss of both first and second rows of OHCs. (**III**) Loss of IHC stereocilia and poor coupling of first row OHC stereocilia to tectorial membrane. (**IV**) Loss of all three rows of OHCs. (Results from different combinations of kanamycin and acoustic trauma in the cat, adapted from Liberman & Dodds, 1984.)

1.6. AMINOGLYCOSIDE OTOTOXICITY

1.6.1. IATROGENIC EXPOSURE

Aminoglycoside antibiotics are derived from various species of *Streptomyces* and *Micromonospora*, or are produced synthetically. Aminoglycosides are used therapeutically as antibiotic agents, which can cause ototoxicity as a side-effect when injected (intravenously / i.v., intramuscularly / i.m., or subcutaneously). Conversely, oral ingestion or topical application of aminoglycosides does not usually result in toxicity; as only 0.6 to 3 % of the administered dose is absorbed (Last & Sherlock, 1960), unless trauma provides a more direct route into the systemic circulation (as demonstrated with an ulcer wound by Kelly et al., 1969, and thermal burns by Dayal et al., 1975).

1.6.2. DRUG CATEGORISATION

A diverse array of different aminoglycosides exist. Resistance to the bactericidal effect of an individual aminoglycoside can result from the acquisition of plasmids containing genes that encode aminoglycoside metabolising enzymes. Thus, a wide variety of aminoglycoside antibiotics have been developed for therapeutic use to combat the problem. Aminoglycoside antibiotics are usually separated by structure into four main groups, typified by: streptomycin; gentamicin; kanamycin; and neomycin (Table 1.6.). (For a comprehensive review see Miller, 1985.)

MAIN CATEGORY	PRIMARY DERIVATIVES	SECONDARY DERIVATIVES
STREPTOMYCIN (vestibulotoxic)	Dihydrostreptomycin (cochleotoxic)	
GENTAMICIN ($^2/_3$ vestibulotoxic and $^1/_3$ cochleotoxic)	Sisomicin	Netilmicin (low ototoxicity)
KANAMYCIN (highly cochleotoxic, no longer in regular use)	Amikacin (cochleotoxic)	
	Dibekacin (vestibulotoxic)	
	Nebramycin ($^2/_3$ vestibulotoxic and $^1/_3$ cochleotoxic)	Tobramycin ($^2/_3$ vestibulotoxic and $^1/_3$ cochleotoxic)
NEOMYCIN (highly cochleotoxic, use now restricted to topical applications)	Paramomycin	
	Ribostamycin	Framycetin

TABLE 1.6. The main structural categories of aminoglycoside antibiotics with the most common derivatives and the corresponding preponderance in ototoxic effect (where known).

1.6.3. THERAPEUTIC APPLICATIONS

Aminoglycosides are usually the antibiotics of first choice in the treatment of serious gram-negative infections, or for prophylaxis of sub-acute bacterial endocarditis (see the British National Formulary, 1996 for recommendations of use). Common patient groups include those with: septicaemia; neonatal sepsis; biliary-tract infections; acute pyelonephritis; prostatitis; and, pulmonary infections (in cystic fibrosis). Gentamicin, tobramycin and amikacin have a wide spectrum but are inactive against anaerobes and have poor activity against haemolytic streptococci and pneumococci, and so are often used in conjunction with a penicillin derivative and / or metronidazole. Netilmicin has a broader spectrum, but is less effective against *Pseudomonas aeruginosa*. Gentamicin is the most commonly administered aminoglycoside, as analysis of serum levels is more readily available for monitoring therapy. Therapeutic doses are: 0.7-1.7 mg / kg / 8 hours i.v. for gentamicin and tobramycin; 7.5 mg / kg / 12 hours i.v. for amikacin; and, 2-3 mg / kg / 12 hours i.v. or i.m. for netilmicin. Neomycin is administered orally at 1 g / 4 hours for bowel sterilisation prior to surgery. Rarely, streptomycin is administered i.m. at 1 g daily for resistant strains of *Mycobacterium tuberculosis*. The important side-effects are ototoxicity and to a lesser degree nephrotoxicity and neuromuscular blockade. As a consequence of potential toxicity, all aminoglycoside antibiotics are contra-indicated by pregnancy and myasthenia gravis. Also, aminoglycoside use should be avoided for long periods, and in patients receiving loop diuretics or with existing renal failure.

1.6.4. INCIDENCE OF TOXICITY

The incidence of ototoxicity has been reported to be between 5 and 14% for amikacin, gentamicin, and tobramycin, and between 2 and 3% for netilmicin (Smith et al., 1977; Matz & Lerner, 1981; Kahlmeter & Dahlager, 1984; reviewed by Scott & Griffiths, 1994). Neomycin and kanamycin are thought to be the most ototoxic aminoglycosides, with

amikacin, gentamicin / tobramycin and netilmicin being of successively decreasing ototoxicity. However, there are confounding factors which restrict the accurate assessment of ototoxic incidence: hearing loss may develop as a result of illness and not therapy (Davey et al., 1982); kanamycin, amikacin and streptomycin are commonly administered at 2 to 3 fold the recommended dose for gentamicin, tobramycin or netilmicin (Edson & Terrell, 1987); and, inconsistency in the definition of what constitutes ototoxicity in terms of level (Scott & Griffiths, 1994), or frequency of deficit (Wood et al., 1996). Cochleotoxicity is reversible between one week and six months after discontinuing gentamicin treatment in 50% of affected patients (Esterhal et al., 1986).

1.6.5. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

Aminoglycoside antibiotics are composed of amino sugars attached to an aminocyclitol ring (hexose nucleus) by glycosidic bonds. The structures are all highly polar, water soluble, cations. Therefore, most of an injected aminoglycoside antibiotic is eventually excreted unchanged through the kidneys (Brummett, 1980). However, accumulation of the drug alone is not proportional to toxicity and the onset of ototoxicity is delayed with respect to aminoglycoside exposure (Brummett, 1980). Gentamicin is probably the most commonly used aminoglycoside antibiotic and has been intensively studied (Begg & Barclay, 1995). *In vitro* exposure of OHCs to gentamicin alone does not result in toxicity. However, metabolic activation by liver enzymes (Huang & Schacht, 1990) and by cochlear lateral wall tissues (Cran & Schacht, 1996) causes toxicity to hair cells. Hence, aminoglycoside ototoxicity could be considered to be a phenomenon of local activation. Furthermore, glutathione can decrease aminoglycoside toxicity *in vitro* (Garetz et al., 1993; 1994), though modulated by nutritional status (Lautermann et al., 1995). Therefore, the concentration of any hypothetical reactive intermediate (cytotoxic metabolite) must be low, and the enzymes involved in activation are unknown, but are most likely to be mixed

function oxidases / cytochrome P450s and / or amine oxidases within the cochlea.

1.6.6. COCHLEAR UPTAKE KINETICS

Early measurements of aminoglycoside accumulation in the cochlea focused on perilymph concentrations (probably due to relative ease of access) and did not reflect on the differences that could affect fluid and tissue distributions (e.g. membrane permeability, cellular transport mechanisms, cellular metabolism). For example, in patients receiving gentamicin therapy, the mean concentration in perilymph has been reported to be 0.84 μg/ml with normal renal function, but significantly higher at 2.45 μg/ml with acute renal failure (Lerner et al., 1982). However, aminoglycoside levels in perilymph have also been found not to correlate with the onset of pathological changes in sensory cells (in the guinea pig; Brummett & Fox, 1982). Furthermore, a more detailed pharmacokinetic study using radioimmunoassay revealed that plasma serum levels always exceed perilymphic fluid concentrations of gentamicin (in the rat; Tran-Ba-Huy et al., 1986); suggesting that true accumulation does not occur. Indeed, the early studies that proposed an active uptake mechanism of aminoglycosides into cochlear fluids have now been convincingly undermined (Henley & Schacht, 1988). Furthermore, gentamicin has been shown to reach a plateau concentration in perilymph within a few hours (Tran-Ba-Huy et al., 1986), and to occur in OHC lysosomes 6 days after i.v. exposure and prior to the development of ototoxicity (in the guinea pig; Hiel et al., 1992). In addition, gentamicin has a slow clearance from the organ of Corti, with a half-life of 7.3 days after 15 μg/min i.v. for 3 hrs, or 36.3 days following 100 mg/kg i.m. daily for 30 days (Tran-Ba-Huy et al., 1986). It is therefore probable that aminoglycosides enter the endolymph via the blood supply of the stria vascularis and are taken up and retained by OHCs. Thus, the selective toxicity of aminoglycosides is no longer attributed to selective tissue penetration, but rather to putative selective

cellular penetration and delayed elimination. In the future, further technological developments (such as in immunohistochemistry) should allow resolution of any remaining controversy over the accumulation of aminoglycosides in the cochlea and the resulting ototoxicity.

1.6.7. DOSE-RESPONSE & POSSIBLE MOLECULAR MECHANISMS OF ACTION

The degree of toxicity depends on the balance between toxin synthesis and detoxification. Toxicity can be either acute or chronic, depending on the type of aminoglycoside, dosage and duration of exposure.

There is some unconfirmed clinical evidence for an acute deficit in cochlear function during tobramycin therapy (Ramsden, 1981) and possibly during gentamicin therapy (Keene & Graham, 1984).* Acute toxicity is considered to correspond with compromised efficiency and to be reversible. The cause of decreased functional performance is thought to be due to antagonism and blockade of calcium ion channels, primarily at the OHCs (Takada & Schacht, 1982). Postsynaptic actions of excitatory amino acids such as glutamate may also be blocked, primarily at IHCs (Schacht, 1993).

All types of aminoglycoside can cause chronic toxicity (Miller, 1985). Chronic toxicity is considered to correspond with cell death and be irreversible. In animal models, chronic doses have been 100-200 mg / kg / day for 7-30 days (depending on aminoglycoside, species and administration route; Miller, 1985). Probably the most widely accepted mechanistic explanation for chronic aminoglycoside ototoxicity is the three stage model proposed by Schacht (1986; 1993). Firstly, the positively charged aminoglycoside molecules may interact with the

---

* The study by Keene and Graham (1984) contradicts the findings - with respect to gentamicin - of the study by Ramsden (1981). Nevertheless, these are both small scale transtympanic electrocochleography studies: confounded by lack of matched controls for comparison; and, limited by an unquantified low level of reproducibility for repeated measurements.

negatively charged glycoconjugate coating on the cell membrane; causing the stereocilia to fuse. Secondly, aminoglycosides are taken up by hair cell membranes by binding to membrane bound phosphatidylinositol-4,5-bisphosphate. The subsequent structural changes in the membrane increase permeability and allow aminoglycosides into the cell. Thirdly, once aminoglycosides are inside the hair cell, the phosphoinositide system - which is known to act as a second messenger from other cells and control intracellular metabolism (Schacht & Zenner, 1986) - is disrupted further; interfering with ribonucleic acid metabolism, protein synthesis, and carbohydrate metabolism. Also, the amount of arachidonic acid available for cell regulation is reduced, and moreover, ornithine decarboxylase would be inhibited, further decreasing cellular compensatory responses. High phosphoinositol metabolism is therefore believed to be the reason for OHCs being the primary target.

More recently, an excitotoxic mechanism involving N-methyl-D-aspartate (NMDA) receptors in the cochlea has been proposed to mediate aminoglycoside cochleotoxicity (Basile et al., 1996). Firstly, the *in vivo* concurrent administration of NMDA antagonists significantly reduces both hearing loss and damage to cochlear hair cells by aminoglycosides. Secondly, the *in vitro* degree of polyamine-like enhancement of [^{3}H]dizocilpine binding to NMDA receptors by different aminoglycosides, has a high correlation with the degree of cochleotoxicity in humans. How the NMDA receptor-interaction and high phosphoinositol metabolism combine to determine aminoglycoside ototoxicity is unclear, and is an obvious line of molecular research for the near future.

1.6.8. ADVERSE EFFECTS

The structural targets of aminoglycoside ototoxicity have been identified as the hair cells, and - if the exposure is severe - the first order neurons of the spiral ganglion (Johnsson et al., 1980) and the stria vascularis (Backus et al., 1987). The OHCs are affected before the IHCs, and at the base first before progressing apically along the cochlea

(Lundquist & Wersall, 1967). More precisely, the first (innermost) row of OHCs is affected before other rows of OHCs (Quick, 1980). Furthermore, the progression of structural damage has been shown to be preceded by uptake of the aminoglycoside into the hair cells (Heil et al., 1993).

Loss of OHC function may manifest as a reduction in the sharp tuning of the cochlea; followed by loss of IHC function that may eventually manifest as a reduction in the frequency range of the cochlea. Thus, it would be reasonable to expect a decrease in ability to discriminate between frequencies before a decrease in the absolute thresholds of individual frequencies. However, hearing loss with respect to high frequencies and progressing to low frequencies, is often the only change perceived by the patient (Meyers, 1970).

1.6.9. CONFOUNDING FACTORS

There are many variables that can affect toxicity and which need consideration. Any factor that may interact with an ototoxic agent, leading to additivity, synergism, potentiation, or antagonism should be controlled where possible. In particular, potentiation occurs when the same region of the cochlea is exposed to both noise and aminoglycoside antibiotic (Hawkins et al., 1975). Also, there are differences in: aminoglycoside structure-activity relationships; the routes of delivery; and - when considering information from animal studies - species susceptibility. Indeed, animal studies have consistently used grossly elevated doses (25-400 mg / kg / day; Miller, 1985) when compared with therapeutic levels in humans (2-15 mg / kg / day; British National Formulary, 1996). Furthermore, it is important to age-match subjects, as presbyacusis follows the same pattern as that observed with ototoxicity (Fausti et al., 1984 and 1992). Finally, there can also be complex temporal pharmacokinetic interactions between drugs in humans, such as with loop diuretics, which if administered after an aminoglycoside can act synergistically leading to ototoxicity (Brummett, 1980).

1.7. CYSTIC FIBROSIS

Patients with cystic fibrosis (CF) repeatedly attend hospital and are frequently treated with aminoglycoside antibiotics. These patients can be expected to return to hospital after the completion of aminoglycoside therapy, and therefore present a suitable group to study potential ototoxic effects.

1.7.1. AETIOLOGY

CF is an inherited autosomal recessive condition most commonly due to a ΔF508 mutation on the long arm of chromosome 7. The affected gene encodes for plasma membrane chloride ion channels, and its mutation results in abnormally dense muco-viscous secretions, leading to predominantly pulmonary and pancreatic pathology. Approximately one in every 2,500 live births in the UK is subsequently diagnosed with CF. Early diagnosis and implementation of chronic therapy with antibiotics and nutritional supplements, with regular monitoring, has raised maximal life expectancy to about 40 years. The aminoglycoside antibiotics (gentamicin, tobramycin and amikacin) are often used to treat the recurrent pulmonary infections which are common with CF. For a useful short review of CF in both adolescents and adults refer to Mulherin and Fitzgerald (1992), or for a recent and more comprehensive review refer to Hodson and Geddes (1995). No greater incidence of middle ear problems has been found in CF patients when compared with a normal age-adjusted population (Forman-Franco et al., 1979; Cipolli et al., 1993).

1.7.2. AMINOGLYCOSIDE COCHLEOTOXICITY

Improved clinical management of CF patients has lead to enhanced survival, although at the expense of therapeutic side-effects such as hearing loss. There have been numerous clinical reports of those with CF developing hearing loss of cochlear origin in conjunction with

aminoglycoside therapy (e.g. Crifo et al., 1980; Pedersen et al., 1987; Morgan et al., 1988; Mulherin et al., 1991). From the above studies, sensorineural hearing loss can be estimated to occur in 3-30 % of CF patients. The CF patients involved in the studies had received therapeutic doses of: different aminoglycosides (mostly gentamicin, tobramycin and amikacin); a varying number of courses (covering 65 to 148+ days in total); and, varying total exposures (from 23 to 42+ g). However, only routine audiometric testing has been applied to these patients and little attempt has been made to separate any changes in cochlear function due to the CF condition alone. Due to the curtailed life expectancy of those with CF, presbyacusis should not confound auditory evaluation. Nevertheless, age will affect aminoglycoside pharmacodynamics (Siber et al., 1975), as the apparent volume of distribution is higher in children (up to 50% of body water can be interstitial fluid) than adults (about 25% of body water is interstitial fluid). Furthermore, renal clearance of aminoglycosides by those with CF has been shown to be greater (by 6-7%) than for non-CF patients (Finkelstein & Hall, 1979), although that is possibly balanced by the tendency of CF patients to be underweight (lowering the proportion of bodyweight available for drug distribution).

1.8. MEASURING COCHLEAR PERFORMANCE

The functional roles of several cochlear structures are now well established, as is the understanding of the techniques used to define the different aspects of auditory performance associated with specific cochlear components. Sensitivity can be measured by pure tone audiometry, selectivity can be measured by frequency resolution, and OHC function can be measured by oto-acoustic emissions.

In order to more fully appreciate a mechanistic approach to the measurement of cochlear performance, it is necessary to cover in some detail: the information coding of sound stimuli; the functional measures available which should enable identification of the sites of sensory deficit; and, the possible progression of sensory deficit. Finally, implications of the pilot studies conducted prior to commencing the project need to be considered.

1.8.1. THE INFORMATION CODING OF SOUND STIMULI

Sensory stimuli disturb receptor cell equilibria, generating action potentials that lead to nerve impulses. Subsequent convergence and divergence of excitatory and inhibitory links within the neural network enable the formulation of sensory impressions which lead to sensations that are recognised as perceptions. The separation of information from environmental stimuli is restricted to: the detection of proportion by sensory receptors; and, the comparison of differences by central processing. The main factors limiting threshold detection are the levels of spontaneous activity and background noise. (For a general review see Handwerker, 1989.)

Conversion of sound stimuli into information-bearing neural signals depends on coding differences in sensitivity (intensity) and selectivity (frequency) (Moore, 1995). Also, the information available for central processing can be modulated by changes in the gain of the detection system (Zwicker, 1986a,b,c). Pooling of information over a wide area to

improve sensitivity always appears to be at the expense of selectivity, and vice versa (Zimmermann, 1989). Therefore the potential for auditory processing by the cochlea is finite and fundamentally involves a basic dichotomy between absolute thresholds and frequency resolution. Hence, deficits in different aspects of cochlear performance can be represented in terms of changes in the sensitivity, selectivity and OHC contributions to the tuning curve at frequency specific points along the cochlear partition (Liberman & Dodds, 1984; Fig. 1.5.).

1.8.2. SENSITIVITY

Psychophysically, auditory sensitivity is primarily a function of loudness perception and as such, is dependent upon the functioning of the whole auditory pathway (Moore, 1989). The perception of loudness is dependent upon both the intensity and frequency of sound (Dadson & King, 1952; Wheeler & Dickson, 1952). For continuous scales such as intensity and frequency both absolute thresholds and difference thresholds exist. An absolute threshold is a measure of the lowest stimulus level to evoke a response. Whereas, a difference threshold is a measure of the minimum change in stimulus level to evoke a response. The smallest discriminable difference in intensity is normally between 0.5 and 5 dB, depending on the absolute intensities employed (Riesz, 1928). The intensity incident at any one point on the organ of Corti can be directly related to the mean firing rate of individual cochlear nerve fibres, and the number of activated fibres (recruitment) at high levels (Pickles, 1986).

Exposure to extreme stimuli can cause thresholds to shift. Changes in thresholds can be either temporary (TTS) or permanent (PTS), due to compensation and damage respectively. Compensatory mechanisms include post-stimulatory auditory fatigue and auditory adaptation (Hood, 1972); a consequence of which is broadening of the auditory filters. Damage is manifested as loudness recruitment (Fowler,

1936) or pathological adaptation (Jerger & Jerger, 1975), a further consequence of which is a restricted dynamic range.

1.8.3. PURE TONE AUDIOMETRY

Absolute intensity thresholds at discrete frequencies are classically recorded psychophysically as a pure tone audiogram, and using the 'method of limits' (i.e. the lowest level at which 50 % of responses are correct; Levitt, 1971). With a compliant subject, pure tone audiometry gives an immediate measure of auditory sensitivity, which although subjective, is more precise and can cover a wider range of frequencies than objective evoked-response measurements (Ballantyne, 1990). Routine clinical pure tone audiometry records thresholds in dBHL for frequencies from 250 Hz to 8 kHz at octave intervals (British Journal of Audiology, 1981,85,86,89).

For the more detailed study of progressive sensorineural hearing loss, high frequency thresholds can be monitored (e.g. Tange et al., 1985). However, as reference equivalent threshold sound pressure levels are not available for frequencies above 8 kHz, such thresholds must be recorded and compared in dBSPL (e.g. Northern & Ratkiewicz, 1985).

1.8.4. SELECTIVITY

Frequency resolution describes the ability to filter out (on the basis of frequency) one stimulus component from another in a complex stimulus. The organ of Corti can be conceptualised as being equivalent to a compressed array of overlapping band-pass filters with characteristic centre frequencies (Fletcher, 1940). The output of any individual filter depends on: the breadth of the filter of interest; and, the output of adjacent filters. The filter bandwidth can be represented as equivalent rectangular bandwidths (ERBs); a rectangular-shaped area that has the same peak transmission as that filter and which passes the same total power for a white noise input (Fletcher, 1940). Each ERB is 11-17 % of the centre frequency and corresponds to about 0.9 mm in distance along

the organ of Corti; for a normal frequency range from 20 kHz at the basal end to 20 Hz at the apical end (Fletcher, 1940). The smallest discriminable difference in frequency is typically 0.5 to 100 Hz depending on the absolute frequencies employed (Wier et al., 1977).

1.8.5. FREQUENCY RESOLUTION

The ability to resolve differences in frequency can be determined by the masked threshold of a signal probe (Mayer, 1894). Usually, the signal probe is a pure tone, whereas the masker is a band of noise covering a range of frequencies. The degree of masking corresponds to the probe to masker intensity ratio and the frequency relationship between the two. The presentation of probe and masker can be either simultaneous using wide masker bandwidths, or non-simultaneous using narrow masker bandwidths (for a comprehensive review see Moore, 1989).

Simultaneous masking corresponds to the swamping (Greenwood, 1961) and / or suppression (Delgutte, 1988) of neural activity. There are two processes conveying information: total neural activity in neurons with different characteristic frequencies (place information; Zwicker, 1970); and phase-locking of nerve impulses to stimulation (possible below 4-5 kHz; Ruggero et al., 1986). Methods typically employed in simultaneous presentations of probe and masker include: psychophysical tuning curves (Christovich, 1957; Patterson & Moore, 1986); notched noise (Patterson, 1976); rippled noise (Houtgast, 1977); co-modulation masking release (spectro-temporal pattern analysis; Hall et al., 1984); and profile analysis (Green, 1988). Confounding factors to be considered when using simultaneous masking methods are off-frequency listening (Johnson-Davis & Patterson, 1979; Patterson & Nimmo-Smith, 1980) and the formation of combination tones (Greenwood, 1971).

Non-simultaneous masking corresponds to the persistence of neural activity whilst revealing the effects of lateral suppression (Houtgast, 1972). As the band-pass filters of the cochlea are relatively

narrow, non-simultaneous masking should provide a more accurate reflection of the neural representation of auditory stimuli. However, if the masker is presented prior to the signal (backward or pre-stimulatory masking), learning processes can have a substantial effect. If the masker is presented after the signal (forward or post-stimulatory masking), adaptation and fatigue can similarly bias the outcome (Duifhuis, 1971).

Only psychophysical tuning curves appear to have been used clinically to show the adverse effects on frequency resolution of ototoxins (salicylates by Bonding, 1979; and, quinine by Karlsson et al., 1991). However, a simplified version of the notched-noise method has been proposed (Stone et al., 1992) for the clinical measurement of frequency resolution; although at present notched-noise measurements only appear to have been applied to patients suffering from noise-induced hearing loss (Bergman et al., 1992).

1.8.6. NOTCHED-NOISE

Auditory filter shapes can be estimated for different frequencies by: the difference in perceptual threshold between a pure tone at a specific centre frequency (f_0) discriminated from surrounding un-notched masking noise; and centred masking noise with different notch widths (Fig. 1.8.6.). Mathematical models for the frequency-response characteristic of the auditory filter (Patterson et al., 1982) can be used to calculate: a symmetrical rounded exponential function for the slope (p); proportionality constant (K); and ERB (as defined in 1.8.4.). The value of p is a representation of the dynamic range for the cochlear filter; where p can be calculated from an equation (see methods 2.4.3.) of the relationship between the pure tone thresholds under various notched and un-notched masking conditions. K represents the retrocochlear efficiency of the detection process; where K can be positive or negative with respect to the spectrum level of the un-notched noise level (a measure of signal-to-noise ratio in dB at the filter output). Therefore, K can reveal frequency-independent psychological effects such as learning or fatigue.

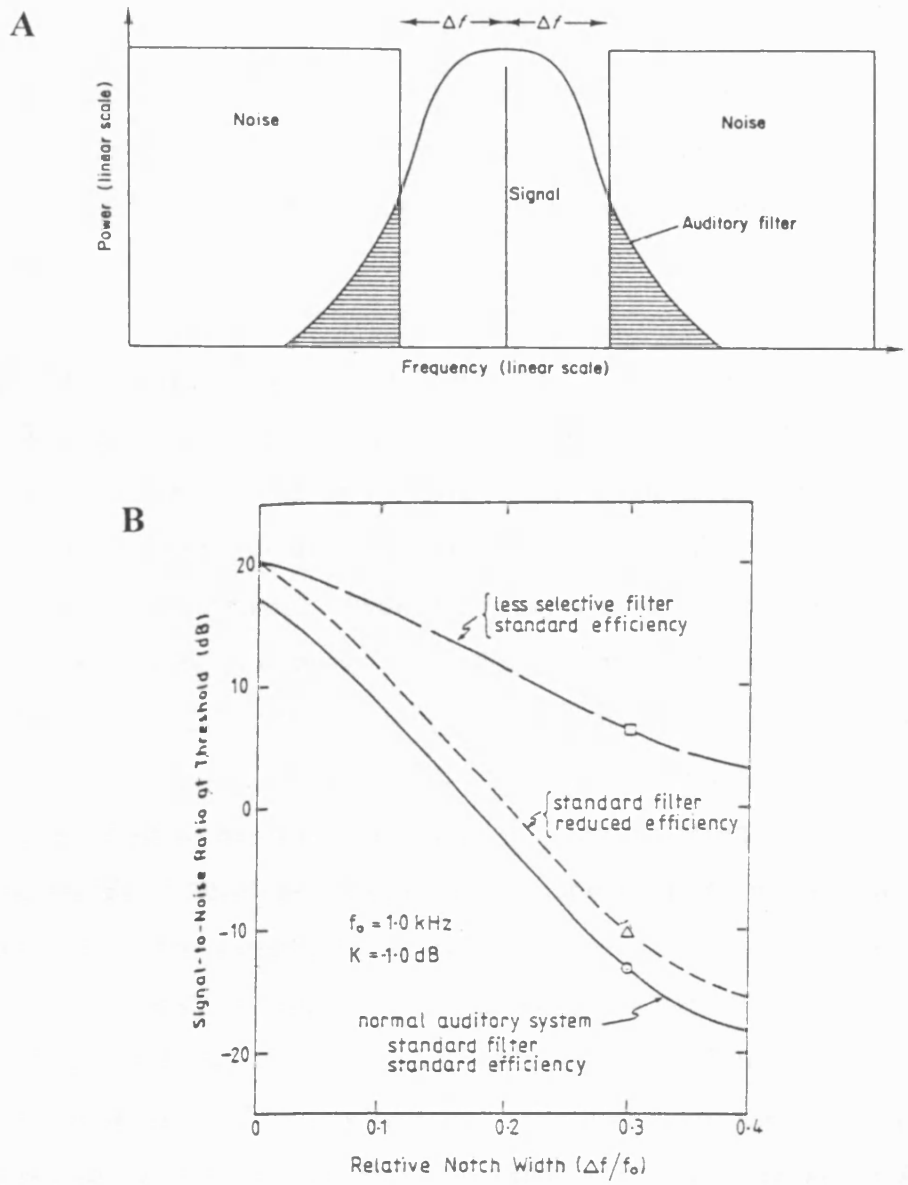


FIG. 1.8.6. Frequency resolution by notched-noise method. (**A**) Schematic illustration of the technique used by Patterson (1976) to determine the shape of the auditory filter. The pure tone threshold is measured as a function of width of the spectral notch in the noise masker. The amount of noise passing through the auditory filter centred at the pure tone frequency is proportional to the shaded areas. (Adapted from Moore, 1989.) (**B**) Illustration of threshold curves produced when a pure tone is masked by a notched noise. The notch is centred on the pure tone (f_0) and the total notch width is $2.\Delta f$ Hz. The solid curve shows how threshold drops as the notch is widened for a normal, young listener (ERB = 0.13 f_0, K = -1.0 dB). The broken line shows the curve for a hypothetical patient with a broad filter but standard processing efficiency (ERB = 0.26 f_0, K = -1.0 dB). The dashed line shows the curve for a hypothetical patient with a standard filter but reduced processing efficiency (ERB = 0.13 f_0, K = +2.0 dB). (Adapted from Patterson et al., 1982.)

The ERB is a representation of filter width (as defined in 1.8.4.), where ERB is equivalent to $(4.p^{-1}).f_0$. Thus, it is possible to derive three measures of tuning integrity at frequency-specific locations within the cochlea.

1.8.7. OHC FUNCTION AND OTO-ACOUSTIC EMISSIONS

The activity of OHCs can 'inject' energy into the travelling wave, sharpening the resonance point, and lowering the threshold for individual characteristic frequencies by up to 40 dB (Zwicker, 1986a,b,c). Furthermore, the complex resonance response of the travelling wave in the cochlear partition grows non-linearly near its peak due to the saturation of OHC function (Johnstone et al., 1986). Changes in the electromotile activity of the OHCs can be measured as oto-acoustic emissions (OAEs) in the ear canal following retrograde transmission (Kemp, 1978). Therefore, OAEs can be used to monitor the sharp tuning component of the transduction process. (For a short or long review see Probst et al., 1991 or Robinette & Glattke, 1997 respectively.)

OAEs can be classified as spontaneous (SOAEs) or stimulus-evoked (EOAEs). SOAEs reflect the continuous oscillation of OHCs in response to excessive gain (Kemp, 1981). Stimulus-evoked OAEs can be: transient (TOAEs / TEOAEs) in response to interrupted broad band stimuli such as clicks (Kemp, 1978); distortion-product (DPOAEs) in response to two simultaneous stimuli such as tones of differing frequency (Kemp, 1979); or stimulus-frequency (SFOAEs) which represent cancellation and addition residuals measured in response to a continuous tone (Wilson, 1980).

The practical application of OAE measurements have been discussed at length elsewhere (Kemp et al., 1986; 1990). OAEs have the advantages of being: non-invasive; objective (requiring no voluntary response from the patient); quick and easy to perform; and, normally highly stable over time (yet sensitive to auditory insult). Furthermore, changes in OAE responses can be measured prior to an individual being

able to perceive any deficit in hearing. However, OAEs have the disadvantages of: being indirect measurements via the middle ear; the possibility of being masked by stimuli; and, at present insufficient human data is available to fully evaluate clinical efficacy. Nevertheless, OAEs are used in clinical screening for the early detection of sensorineural loss which correlates with cochlear mechanical loss (Martin et al., 1990). The presence of OAEs indicates normal auditory function up to and including OHCs. Either a defective generating mechanism (sensory loss), or a defective transmitting mechanism (conductive loss), would be manifest as reduced OAEs (Patuzzi, 1993). Therefore, OAEs provide a very useful tool for research into mechanisms of auditory function / dysfunction.

1.8.8. CONFIRMATION OF OAE ORIGINS

Some researchers have questioned the cochlear origin of OAEs and proposed changes in middle ear impedance as an alternative and exclusive cause of OAEs (Stephen & Badham, 1996). However, there is substantial evidence to confirm OAEs originate from a biological process generating mechanical activity in the cochlea. OAEs are physiologically vulnerable: DPOAEs decrease with increasing age (Lonsbury-Martin et al., 1991); exposure to excessive noise (TTS; Puel et al., 1995); and anoxia (Kim et al., 1980); SOAEs decrease with anaesthesia (Probst & Beck, 1987); and no OAEs are measurable from 'dead' ears (Kemp, 1979). Various ototoxic drugs have also been shown to affect OAEs: aspirin reversibly abolishes SOAEs in humans (McFadden & Plattsmier, 1984); also in humans TOAEs can be lowered by quinine (Karlsson et al., 1991) and cis-platin (Zorowka et al., 1993); and, gentamicin lowers DPOAEs in guinea pigs (Brown et al., 1989), and TOAEs in humans (Holtz et al., 1994). Indeed, the study of ototoxic drugs has also revealed important differences between the generation mechanisms for SOAEs and DPOAEs (using aspirin in humans; Wier et al., 1988).

OAEs are thought to be coincident with the sharp tuning and high sensitivity of the transduction process in the cochlea (Kemp, 1979). OAEs exhibit sharp tuning; as equal suppression contour-maps have the same shape with change in frequency-response characteristics, as basilar membrane tuning curves (Brown & Kemp, 1984). High sensitivity is exhibited; as OAEs are not present with severe sensorineural losses, which correspond to a maximum contribution by OHCs to sensitivity of 40 dB.* Hence, vibrational amplification powered by the metabolism of the cochlea is the only viable explanation for the sensitivity and frequency selectivity observed, which is greater than is credible by passive physics alone (Zwicker, 1986a,b,c; Brass & Kemp, 1993).

OAEs have a strong non-linear component, as: OAEs tend to be greatest between 250 Hz and 2500 Hz (probably as middle ear transmission is most efficient in this range); and EOAEs with varying stimulus intensity saturate in growth (Kemp, 1979). EOAEs also have long latencies, suggesting an origin at some time after the presentation of stimuli. EOAE responses are delayed by 5-15 ms after stimuli presentation, and thus are too late for middle ear 'echoes' (Kemp, 1978).

Evidence relating to pre-synaptic generation suggests the OHCs are the origin of OAEs: OAEs are not the result of the middle ear muscle reflex (Wilson, 1980; Anderson, 1980); the amplitude of DPOAEs can be affected by stimulation of the olivocochlear bundle (Mountain, 1980); furthermore, OAEs can be evoked by bone conduction (Rossi & Solero, 1988) and altered by contralateral stimulation (Collet et al., 1990); finally, SOAEs indicate the ear is capable of generating discrete frequency sounds (Glanville et al., 1971).

1.8.9. SPONTANEOUS OTO-ACOUSTIC EMISSIONS

SOAEs may be the most sensitive indicators of sensorineural hearing loss, though only when and where they are present (Moulin et

---

* i.e. OAEs are absent if there is a loss of 40 dB or more, and that implies a 40 dB contribution by the OHCs.

al., 1991). SOAEs can be either 'true' (SOAEs), or click-synchronised (CSSOAEs) discrete frequency emissions. By sealing a microphone in the ear canal, SOAEs can be measured effectively for frequencies between 0 and 12 kHz, down to intensities as low as 0 dBSPL or more. By sealing a microphone and speaker in the ear canal, CSSOAEs can be measured by presenting a click stimulus every 80 ms. Following each click the first 20 ms contains a large transient-evoked component that is discarded. CSSOAEs can be measured effectively for frequencies between 0 and 6 kHz, down to intensities as low as -30 dBSPL (Wable & Collet, 1994). There are currently two hypotheses to explain the origin of CSSOAEs. It is possible that the clicks may jolt susceptible OHCs into action and hence provide an exaggerated reflection of SOAEs. However, when directly compared with the SOAE method, although the CSSOAE method has been shown to significantly increase the number of recordable discrete frequency emissions per ear, the amplitude of these CSSOAEs is significantly smaller than SOAEs (Burr et al., 1997). Therefore, it has been suggested (D.T. Kemp *pers. comm.*, 24 September 1996) that any spontaneous oto-acoustic emissions present may be repeatedly suppressed by the clicks and re-emerge after each click in synchrony within the remaining 60 ms time window. Thus, CSSOAEs are thought to represent a decaying transient-evoked component combined with a recovering spontaneous component.

1.8.10. DISTORTION PRODUCT OTO-ACOUSTIC EMISSIONS

DPOAEs can be measured in all individuals with normal hearing, and also in the presence of a more severe hearing loss than with SOAEs (Wier et al., 1988). DPOAEs are evoked in response to two simultaneous pure-tones of different frequencies, i.e. the combination tones: at frequencies f_1 and f_2 (typically separated by an optimum ratio of 1.22; Harris et al., 1989); and levels l_1 and l_2 (typically both at an optimal

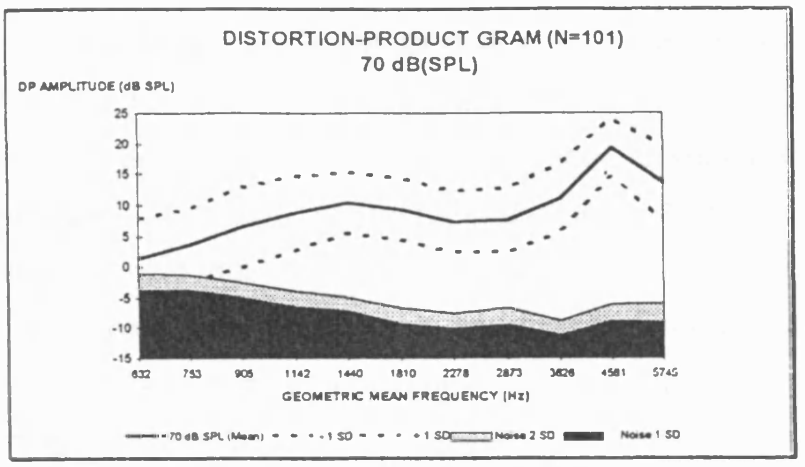
intensity of 70 dBSPL; Hauser & Probst, 1989; Sun et al., 1995*). Inherent non-linearity generates a third tone at $2f_1$-f_2 (and other distortion-products at $3f_1$-$2f_2$, $4f_1$-$3f_2$, $2f_2$-f_1, $3f_2$-$2f_1$ and $4f_2$-$3f_1$), the inter-modulation implies a presence as a mechanical disturbance probably as a travelling wave on the basilar membrane (Martin et al., 1987). However, the frequency closest to the site of DPOAE generation may move from near f_2 to near $2f_1$-f_2 with increasing stimulus levels (Brown & Kemp, 1983,84), though the exact nature of the generation sites is still uncertain (He & Schmiedt, 1993). The amplitude of DPOAEs can be presented as a function of: f_1 (the lower / primary tone); f_2 (the higher / secondary tone); $[f_1 + f_2] / 2$ (the mean frequency); $2f_1$-f_2 (the distortion-product frequency); or, the square root of $[f_1 \times f_2]$ (the geometric mean frequency). Nevertheless, DPOAEs are emitted with specific product terms in relation to the two stimulus tones, and have the potential to give a level and frequency-specific indication of the degree of hearing loss (Gaskill & Brown, 1990; Nelson & Kimberley 1992).

DP-grams (distortion product audiograms) can be recorded by keeping the intensity of the two tones constant and varying the frequency of each. The shape and level of DP-grams vary between ears, individuals and distortion product stimulus parameters, but all individuals with normal hearing appear to follow the same pattern (Fig. 1.8.10.A.; Vinck et al., 1996). DPOAE levels tend to increase with increasing frequency, except for a trough around the mid-frequencies (2-4 kHz); and can follow changes in sensitivity (approximately correlating with changes in pure tone audiometry thresholds; Gaskill & Brown, 1990). Alternatively, distortion product input-output functions (growth rates) can be recorded by keeping the frequency of the two tones constant and varying the intensity. The shape and threshold of input-output functions also vary between ears, individuals and distortion product stimulus parameters (Fig. 1.8.10.B. A-F; Nelson & Kimberley 1992).

---

* Although apparently published, the abstract by Sun et al. (1995) can not be obtained via the British Library.

A



B

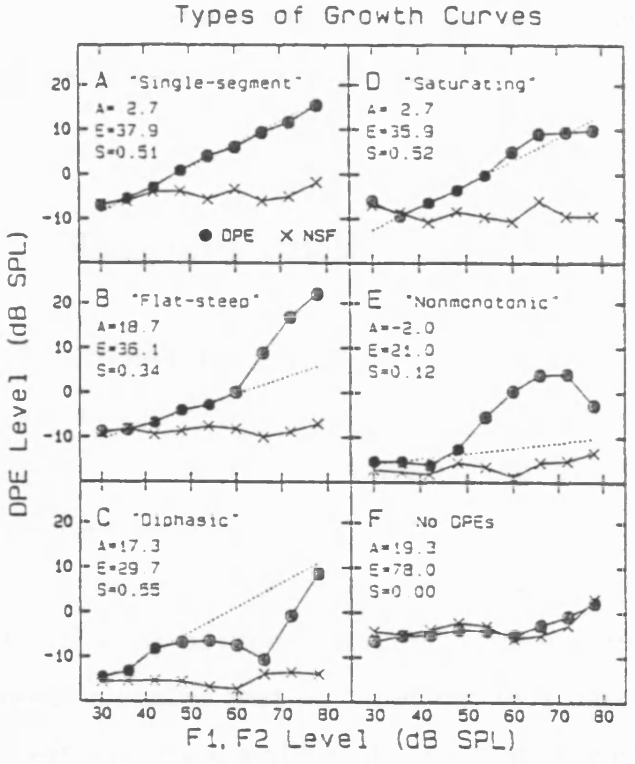


FIG. 1.8.10. Distortion product oto-acoustic emissions. (**A**) Typical DP-gram. Mean $2f_1$-f_2 level plotted against stimulus frequency for 101 normal ears (adapted from Vinck et al., 1996). Where, I_1 and I_2 = 70 dBSPL, and f_1:f_2 ratio = 1.22. (**B**) Categories of distortion product input-output function shape. In the examples shown (adapted from Nelson & Kimberley, 1992) the highest stimulus level used was 80 dBSPL, and the stimulus level at which the DPOAE response crossed the mean noise floor was taken as a measure of 'threshold'. Filled circles (DPE) indicate the points used to extrapolate a best-fit line (dotted) to the noise floor (NSF). Where: A = auditory threshold in dBSPL; E = DPOAE 'threshold'; and, S = slope of the best-fit line.

Shapes incorporating dips in the DPOAE output with increasing stimulus input (Fig. 1.8.10.B. C) could be generated by either: phase cancellation between two or more acoustic components; or, superposition of two level-dependent distortion product components (Probst et al., 1991). Importantly, if two out of phase generator sites are contributing to a DPOAE and one site is damaged, a dip could become a peak. Thus, an increase in DPOAEs prior to a decrease may not necessarily be due to an overloading increase in cellular activity, but rather a gradual loss of generator sites. Therefore, although DP-grams are useful to quickly evaluate hair cell function over a range of frequencies, input-output functions are necessary to monitor changes more carefully at individual frequencies.

1.8.11. FACTORS CONFOUNDING MEASUREMENTS OF COCHLEAR FUNCTION

All tests should be non-invasive due to involvement of human subjects; and as the cochlea is inaccessible, it is often necessary to resort to subjective instead of objective tests of auditory function. When subjective tests are employed it is important to distinguish between tests of memory (i.e. discrimination, e.g. alternative forced choice: Yes / No; or 1st / 2nd) and tests of modulation detection (i.e. resolution). Similarly, there is a need to ensure that no other stimulus parameter is being used as a cue to perform any specific task. Another important realisation with the use of psychophysical techniques is that there may be confounding neurotoxic effects. Furthermore, normal middle ear function is assumed and is particularly important to establish with OAE measurements (Kemp, 1981; Kemp et al., 1990).

1.8.12. POSSIBLE PROGRESSION OF SENSORY DEFICIT IN OTOTOXICITY

Any progressive damage to the structure of the cochlea must be manifest as a gradual loss of functional ability in stages. With

ototoxicity, high frequencies (transduced at the base of the cochlea) are affected before low frequencies (transduced at the apex of the cochlea) (Meyers, 1970). Therefore, all tests should incorporate some aspect of this high to low frequency progression in deficit.

However with aminoglycoside ototoxicity, OHC loss prior to IHC (and neuron) loss, should cause a disarrangement of OAEs before any affect on overall sensitivity. Furthermore, it is plausible that frequency selectivity will be affected before sensitivity (Pratt & Comis, 1982; Puel et al., 1987), due to the relative functional contributions of OHCs and IHCs. Similarly, DPOAEs can be expected to be affected prior to pure tone sensitivity (Mulheran & Degg, 1997; Katbamna et al., 1998*). To date there appears to be no experimental evidence to support OAEs being affected before frequency resolution (or vice versa); although it may be reasonable to expect objective OAE measurements to detect a change before subjective psychophysical frequency resolution measurements. Thus it follows that the anticipated progression of detecting change is: OAEs $\geqslant$ frequency resolution > PTA (as OHCs are affected before IHCs).

Although the basal end of the cochlea is damaged first, psychophysical testing at high frequencies can be expected to be highly variable (due to more difficult perception and greater effect of insert earphone / headphone placement on coupling efficiency). Hence, any deficits in auditory performance may be detected earlier at mid-frequencies. Finally, older patients who have received more aminoglycoside therapy may be expected to exhibit greater chronic changes in auditory function due to a greater exposure. Likewise, younger patients who have received less aminoglycoside therapy may be expected to exhibit greater acute changes in auditory function due to the greater potential for loss.

---

* KATBAMNA B., HOMNICK D.N. & MARKS J.H. (1998) Contralateral suppression of distortion product otoacoustic emissions in children with cystic fibrosis: effects of tobramycin. Journal of American Academy of Audiology, 9 (3):172-178.

1.8.13. PILOT STUDIES

The preliminary findings of research carried out by Degg and Mulheran in 1995 indicated that CF patients on chronic gentamicin therapy at Leicester exhibited elevated DPOAE iso-thresholds at 2 and 4 kHz (but not at 6 kHz). Furthermore, one from the total of 15 CF patients had profound bilateral high frequency hearing impairment when tested with PTA. They proposed that DPOAEs could be used to indicate a sub-clinical effect of aminoglycosides on OHC function that precedes any alteration in auditory perception (Degg, 1995; Degg and Mulheran, 1996; Mulheran et al., 1996; Mulheran & Degg, 1997).

METHODS

For a comprehensive list of equipment and corresponding specifications necessary to reproduce the experimental work contained herein see appendix 6.2.. First of all it is necessary to stipulate the test facilities and background noise. Factors used for the inclusion / exclusion of test subjects are then detailed. The use of a battery of tests on these subjects was used to reveal any changes in different aspects of cochlear performance. The test battery comprised: otoscopy; tympanometry; pure tone audiometry (including high frequencies from 10-16 kHz); frequency resolution (by notched noise method); and oto-acoustic emissions (both spontaneous and distortion product). The calibration, stimulus-recording parameters, assumptions and implementation of each test is detailed. Finally, the specific hypothesis being tested by the study is considered and the methods of analysis justified.

2.1. TEST LOCATIONS

Recordings were made at four separate hospital sites: Leicester Royal Infirmary (LRI); Northampton General Hospital; Peterborough District Hospital; and, Birmingham Heartlands Hospital. At LRI all recordings were performed in a double walled, electrically screened audiology test room. However, at the other (peripheral) sites the tests could only be performed in a relatively quiet side-room. All background noise recordings were made under conditions similar to the test sessions (i.e. comparable time, equipment on, doors closed, etc.).

The background noise levels in the audiology test room were regularly measured using: dB(A); linear setting; and 1/3 octave-band approach (from 250 Hz to 8 kHz, in octave increments). Measurements were made using a

precision SPL meter Type 2235, with a free-field $^1/_2$" microphone Type 4191, connected to a band-pass filter Type 1618. The background noise levels were also measured when attenuated in each ear canal of the operator (who had no SOAEs) using the ILO92 'spectrum analyser' facility (in dBSPL between 0 and 12 kHz). At visits to peripheral hospitals the background noise could only be measured using the ILO92 'spectrum analyser' facility. Therefore at each separate visit, recordings were made free-field using the ILO92, as well as when attenuated in each ear canal.

2.2. AUDIOLOGICAL SCREENING OF SUBJECTS

All encountered CF patients who were willing to participate were recruited. Control subjects were recruited in order to cover a similar distribution of both age and gender as patients. Importantly, reports of existing hearing problems were not permitted to influence the recruitment of subjects (i.e. in the recruitment of subjects by the researcher, known hearing loss did not bias either: the exclusion of control volunteers; nor the inclusion of patients).

2.2.1. CONTROL VOLUNTEERS

Only individuals fulfilling the following basic inclusion / exclusion criteria were considered to have normal hearing for the purposes of more advanced testing.

Questioning:	No evidence of current hearing problems.
Questionnaire:	No history of hearing-related health problems;
	No evidence of ototoxic drug exposure.
Otoscopy:	No signs of injury or surgery in the ear;
	No obstruction, inflammation, or discomfort in the canal;
	No dullness, pallor, or distortion of the drum.

Tympanometry: Compliance = from 0.3 to 1.6 cm^3

Peak pressure = from -50 to +50 daPa in adults and -150 to +50 daPa in juveniles, in ear under test.

Pure-tone audiometry: Exclude if one frequency exceeds 25 dBHL, or if two or more frequencies exceed 20 dBHL in air conduction threshold, for all frequencies at octave intervals from 250 Hz to 8 kHz, in either ear.

The questionnaire and questioning can justify the exclusion of one or both ears. Otoscopy and tympanometry reflect only the ear tested - as the left and right outer and middle ears can be regarded as independent entities - and therefore an abnormality justifies only the exclusion of that ear. Pure-tone audiometry may reflect a systemic influence - as the whole auditory pathway is being tested - and therefore justifies the exclusion of both ears, even if only one ear fails the above threshold criteria.

2.2.2. CYSTIC FIBROSIS PATIENTS

Patients complying with the control subject selection criteria (except for ototoxic drug exposure) were grouped together. Patients not complying with the control subject selection criteria were considered on an individual basis. Patients and control subjects were tested concurrently.

Patient records were meticulously examined for evidence of ototoxic drug exposure; with particular emphasis on aminoglycoside antibiotics, duration of therapeutic episodes, administered dosages, body-weight and serum assay results (see appendix 6.3. for aminoglycoside / antibiotic dosage form). Assuming differences in serum levels for individual types of aminoglycoside were greater than the differences in toxicity between types of aminoglycoside, all aminoglycoside antibiotics can be combined for the purposes of evaluating lifetime exposure prior to testing. The total aminoglycoside dosage was analysed in terms of: level, duration, and

number of occasions when serum levels were above and below the recommended therapeutic range (Barza & Lauermann, 1978). Based on a rough consensus of clinical experience rather than experimental evidence, serum levels for gentamicin and tobramycin can be categorised as follows: perfect = trough <2 and peak 6-10 mg/l; acceptable = trough 2-3 and peak 5-12 mg/l; potentially toxic = trough >3 and peak >12 mg/l; sub-therapeutic = peak <5 mg/l (R.A. Swann *pers. comm.*, 24 July 1998). Furthermore, at the time of testing each adult patient was questioned specifically with regard to their therapeutic history: "when did you first start intravenous medication; how many courses do you have each year; which hospitals have you attended for treatment; which aminoglycoside(s); what was the course duration, dose and daily frequency; has the aminoglycoside always been diluted; what was the rate of administration; have you ever had any problems associated with intravenous aminoglycoside therapy (e.g. any unusually high serum levels, dizziness or ringing in the ears)". Where there were discrepancies in the records that could not be supported by the information from questioning the patient directly, nor from interpolating duration and dose; it was occasionally necessary to assume courses were for 7 days, 3 times per day, of either 80 mg gentamicin, or 120 mg tobramycin (estimated median therapeutic values). In order to calculate aminoglycoside exposure in mg/kg it was frequently necessary to interpolate (and occasionally necessary to extrapolate) patient bodyweight. Furthermore, where audiological tests were repeated on the same patient, only the most recent test result - representing the highest aminoglycoside exposure - was included.

2.2.3. ETHICAL APPROVAL

Prior to any testing, appropriate ethical approval was sought and obtained in writing from the relevant Local Health Authority Research Ethics Committees, and agreement obtained from involved hospital departments and collaborating clinical staff. In addition, informed consent was obtained from each test subject (see appendix 6.4. and 6.5. for information sheet and consent form respectively). For full-time research to be carried out at Leicester Royal Infirmary it was necessary to obtain a contract as an honorary member of the Medical Physics department (Electro-Diagnostics). When testing any subject under the age of consent (16 years) unwritten hospital policy required another adult to be present to safeguard against allegations of misconduct.

2.2.4. HEARING TEST QUESTIONNAIRE & QUESTIONS

Every subject completed a questionnaire (see appendix 6.6.) to establish background medical history, including evidence of any hearing-related health problems or of potential ototoxic drug exposure. Furthermore, preceding each test session the subject was questioned concerning any hearing related problems (e.g. presence of a cold) and any medication taken recently (e.g. aspirin). Finally prior to undertaking each test, the requirements of the procedure were explained to the subject.

2.2.5. OTOSCOPIC EXAMINATION

At each test session otoscopy was performed to exclude: signs of injury or surgery in the ear; obstruction, inflammation, or discomfort in the canal; and dullness, pallor, or distortion of the drum. The normal appearance of the tympanic membrane could thus be distinguished from that seen in cases of: tympanosclerosis, secretory otitis media (glue ear), acute otitis media, retraction, cholesteatoma, and perforation. For an up to date review of otoscopic technique see Carney & Bicknell (1995).

2.2.6. TYMPANOMETRY

Tympanometry was employed in order to verify normal middle ear transmission characteristics. Conventional tympanometry tests the response of the middle ear at only one frequency (226 Hz) at 85 dBSPL (British Journal of Audiology, 1992). Compliance (see appendix 6.1.6.) is measured in response to a changing pressure and expressed as an equivalent volume of air. The pressure at which compliance was maximal was designated as the peak pressure. The tympanometry equipment was calibrated at the start and end of the project in accordance with the *'Specification for instruments for the measurement of aural acoustic impedance / admittance'* (BSEN 61027:1993); with additional monthly references to both 1 cm^3 and 4 cm^3 cavities.

The middle ear function of each ear was always checked at every test session. Before starting tympanometry the subject was told to expect a short tone and a small change in pressure, and asked to try to resist the temptation to swallow. The probe with an appropriately sized sterile tip attached, was carefully inserted into the ear canal and positioned to achieve a sealed fit (as displayed by the instrument). The volume between probe tip and eardrum, and the change in middle ear compliance characteristics for a pressure range from -300 to +200 daPa was then measured.* No ears were excluded on grounds of tympanometry volume, due to variability with selected eartip size and depth of insertion.

---

* Clinically accepted normal values for compliance and peak pressure (see methods 2.1.1.) are reported in the British Journal of Audiology (1992).

2.2.7. ADVANCED HEARING TESTS

Once basic screening had been performed, and a subject selected, a battery of more advanced audiological measurements was carried out at subsequent test sessions as follows:

- High frequency pure tone audiometry; at 10, 12, 14 and 16 kHz.
- Frequency resolution: by the notched-noise method; at 2, 4 and 8 kHz.
- Oto-acoustic emissions: DP-grams; DP input-output functions at 6, 4, 2 and 8 kHz; CSSOAEs and SOAEs.

Where possible, the right ear was tested before the left ear for all tests. Usually, tympanometry was performed at the start of each session and standard PTA was performed in the first session. High frequency PTA thresholds were always assessed in ascending frequency. Frequency resolution at 8 kHz was typically tested following high frequency PTA (using the same inset earphone placement). Frequency resolution at 2 and 4 kHz was not performed in any particular order. OAE tests were performed in the order set out above, except in the left ear where CSSOAE and SOAE tests were performed first (preventing the necessity to change computer programs). Typically three or four 1-2 hour sessions were required with each subject to complete all of the tests. It should be noted that, when presented with a choice of estimates for the threshold of any psychophysical test, only the worst threshold was included (in both controls and patients). The detailed method for each procedure will now be considered in turn.

2.3. PURE TONE AUDIOMETRY

Pure tone audiometry is the standard clinical method used to establish absolute thresholds for a range of individually presented frequencies.

2.3.1. CALIBRATION

All equipment was electrically and acoustically calibrated as detailed below before the first testing of the study commenced. Also, all acoustic calibration values were re-checked after the last testing of the study was performed.

Reference equivalent threshold sound pressure levels (RETSPL) were set in accordance with the *'Specification for standard reference zero for the calibration of pure tone air conduction audiometers'* (BS 2497:1992), and the *'Specification for a standard reference zero for the calibration of pure tone bone conduction audiometers'* (EN 27566:1991). Tolerances for frequency, intensity and harmonic distortion were in compliance with the *'Specification for Audiometers, part 1. pure-tone audiometers'* (BSEN 60645-1:1995).

Calibration of the following set up is traceable to the National Physical Laboratory (NPL) and was used as the reference standard for all other equipment: A precision SPL meter Type 2235 with input stage Type ZC-0200, fulfilling the *'Specification for sound level meters'* (BS 5969:1981); and band-pass filter Type 1618, fulfilling the *'Specification for octave and one-third octave band-pass filters'* (IEC 225:1966); coupled to an artificial ear Type 4152, with a condenser microphone Type 4144, fulfilling the requirements for a *'Provisional reference coupler for the calibration of earphones used in audiometry'* (IEC 303:1970); or an artificial mastoid, fulfilling the *'Specification for an artificial mastoid for the calibration of bone*

vibrators used in hearing aids and audiometers' (BS 4009:1975), as required.

At each calibration session, a Type 4230 piston phone was referenced to the Type 4144 microphone to ensure no drift had occurred in the NPL traceable standard. Furthermore, correction factors for all other microphones were calculated with respect to the Type 4144 standard, using the Type 4230 piston phone.

All standard audiometric frequencies (see methods 2.3.2.) were produced by an audiometer. Also, frequencies of 10000, 12000, 14000 and 16000 Hz were produced (+/- 5 Hz) using a signal generator (2.82 +/- 0.01 Vp-p) and mixed through the audiometer via the external tape input, to Etymotic insert earphones. The earphone under test was inserted into the Zwislocki coupler to a depth of 4-5 mm (as the first impedance determining cavity is 5-6 mm within the coupler). The Etymotic insert earphones have a flat frequency-response; but, the Zwislocki coupler has a frequency-response that can vary +/- 6 dB depending on placement (see manufacturers specifications). In all other respects the calibration was to the same standards as for lower pure tone frequencies (detailed above).

2.3.2. RECORDING PARAMETERS

Air conduction thresholds were recorded in dBHL for 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz. 10000, 12000, 14000 and 16000 Hz were recorded in dBSPL as there were no RETSPL values for frequencies above 8 kHz (ISO 389-5*). Each stimulus presentation would consist of a pure tone of 1 to 3 seconds in duration, with subsequent presentations separated by a 1-6 second interval. The duration of presentations, and

* ISO 389-5 reports dBHL values for frequencies above 8 kHz, however the document is as yet unpublished and so the information is unavailable outside NPL.

interval between presentations was varied in order to prevent responses falling into a repetitive pattern.

2.3.3. ASSUMPTIONS AND LIMITATIONS

Identification of false negative and false positive responses depends on the judgement and experience of the operator, particularly in recognising fatigue and distraction of the subject. Furthermore, the degree of synchronisation of response with stimulus can be used to indicate a true response. A response would be recorded if it occurred within approximately one second of presentation and then only if the response lasted for approximately one second.

The clinical definition of a hearing loss is somewhat subjective, with no universal consensus achieved. Here, an ear was considered to have a hearing loss if one frequency exceeds 25 dBHL, or if two or more frequencies exceed 20 dBHL in threshold, for all frequencies at octave intervals from 250-8000 Hz inclusive. For subsequent pure-tone audiograms a change of 15 dB or greater at any 2 or more consecutive frequencies within the three years of data collection was also considered to constitute a loss.

2.3.4. IMPLEMENTATION OF TECHNIQUE

Every subject received explicit instructions of what to expect: "we will test one ear and then have a break before the other ear; we are looking for the quietest sounds you can hear; we will start reasonably loud so you can know what to listen for and will get quieter; but not to worry if you can't hear the tone for a short period of time - we will be going below your threshold and will work back up again - as we will be going up and down to get an average; only respond if you are sure you can hear a tone, and for as long as you hear a tone; and, we can break at any time if you have any questions".

Following the above instructions, any subject incorrectly responding to 50% or more of the presentations, was considered to be unable to perform the test adequately and the results would be excluded from the study.

Air conduction audiometry:

Positioning of the headphones was done by the operator, and not by the subject, in order to minimise variation in headphone placement (Flottorp, 1995). Thresholds were established for 1 kHz, then each frequency in ascending order to the highest frequency (8 kHz), before the lowest frequencies (250 and 500 Hz) and finally 1 kHz was repeated. If the first and last (1 kHz) thresholds were not within 5 dB of each other, the results for that test session would be excluded from the study. Threshold values +/- 5 dB for individual frequencies were obtained by a modified version of the method of limits, whereby the level of each stimulus presentation was determined by the preceding response of the subject (British Journal of Audiology, 1981).

Establishment of a threshold to 5 dB resolution:

1. For each frequency the first tone was presented at 25 +/- 5 dBHL. If a positive response was not evident at the first presentation, the level of subsequent presentations was increased in 10 dB increments until a positive response occurred.
2. Following the first positive response, presentations descended in 10 dB steps until a missed response, and then ascended in 5 dB steps until a positive response.
3. The process of descending and ascending presentations was repeated, starting at 10 dB above the level of the lowest positive response, until it was possible to assign a threshold as described below.

4. The lowest level to first yield two consecutive positive responses was recorded as a threshold value for that frequency.

High frequency air conduction audiometry:

Prior otoscopy provided guidance for placement of the insert earphones by the operator. The foam ear tip was rolled into the smallest diameter possible, inserted into the ear canal and held in the canal until the foam had expanded. The tip was then withdrawn slightly (by 1-3 mm) to ensure no occlusion against any bends in the canal wall. If the tip fitting was difficult the subject was instructed to report any feeling of discomfort or slippage. The depth of insertion was calculated from a subjective appraisal of the visible length of foam ear-tip protruding from the ear canal. Threshold values +/- 5 dB for 10, 12, 14 and 16 kHz, were obtained as for standard air conduction audiometry (detailed above). After completion of the test the tips were removed and checked to see they were free from wax. If wax blocked the tip the results for that test would be discarded and a new tip would be fitted with a more shallow placement in order to repeat the test.

Bone conduction audiometry:

The operator carefully placed a bone conductor over the mastoid process ensuring no contact with the pinna. The procedure for bone conduction pure tone audiometry (British Journal of Audiology, 1985) was then the same as for air conduction. However, bone conduction thresholds were recorded in dBHL for 250, 500, 1000, 2000, 3000 and 4000 Hz only. Bone conduction was only performed if air conduction indicated a hearing loss and was used to establish if that loss was conductive or sensorineural.

Masking:

Sometimes it was necessary to present narrow band noise continuously to the non-test ear, in order to remove the possibility of the test sound being detected by the non-test ear in cases of unilateral hearing loss (British Journal of Audiology, 1986). Masking was implemented when any one of the following three conditions was met:

1. There was a difference of 40 dB or more in air conduction thresholds between the two ears.
2. The bone conduction threshold was 10 dB or more better than the air conduction threshold of the same ear at the same frequency.
3. The bone conduction threshold was 40 dB or more better than the air conduction threshold of the other ear.

When indicated by fulfilment of one of the above conditions, the pure tone threshold in question would be reassessed with narrow band noise acting as a masker. The masker would be presented using either an insert earphone in the case of bone conduction audiometry, or headphone in the case of air conduction audiometry. First the threshold for the narrow band noise would be established, then the pure tone threshold would be reassessed with narrow band noise at 20 dBSL. The pure tone threshold would then be reassessed with 10 dB increments in masking, until three consecutive thresholds within 5 dB of each other were recorded.

2.4. FREQUENCY RESOLUTION

Frequency resolution is a method used in psychophysical research to establish the frequency selectivity at pre-determined centre frequencies by a simultaneous notched-noise masking paradigm.

2.4.1. CALIBRATION

All equipment was electrically and acoustically calibrated as detailed below before the first testing of the study commenced. Also, all acoustic calibration values were re-checked after the last testing of the study was performed.

Electrical checks for 2, 4 and 8 kHz notched noise were made with the CD signal connected direct to a signal analyser. The CD player was always set to deliver a flat output at the line-output. Each CD track was checked for: centre frequency; frequency widths of notch and both side-bands; and, difference between side-peaks and centre-trough in dBV.

Acoustically, 2 and 4 kHz were performed using TDH-39 headphones, whilst 8 kHz required Etymotic insert earphones. Thus, due to differences in speaker attenuation characteristics there were different calibration procedures for 2 and 4 kHz compared with 8 kHz.

Acoustical checks for 2 and 4 kHz notched noise were made with the CD signal mixed through the audiometer to TDH-39 headphones (coupled to signal analyser via artificial ear, microphone and SPL meter; see Figure 2.4.1.). The audiometer level and tape input gain control were set to deliver 70 +/-2 dBSPL overall at headphones, before checking the: intensity in/of notch using a 1/3 octave filter; intensity of masker side-bands using a linear filter; and, linearity of output over 40, 50, 60, 70 & 80 dBSPL. Also, checked was the spectrum level of masker (53 +/-3 dBSPL/Hz) and relative depth of noise floor (35 +/- 3 dB). Furthermore, the attenuation (filtering

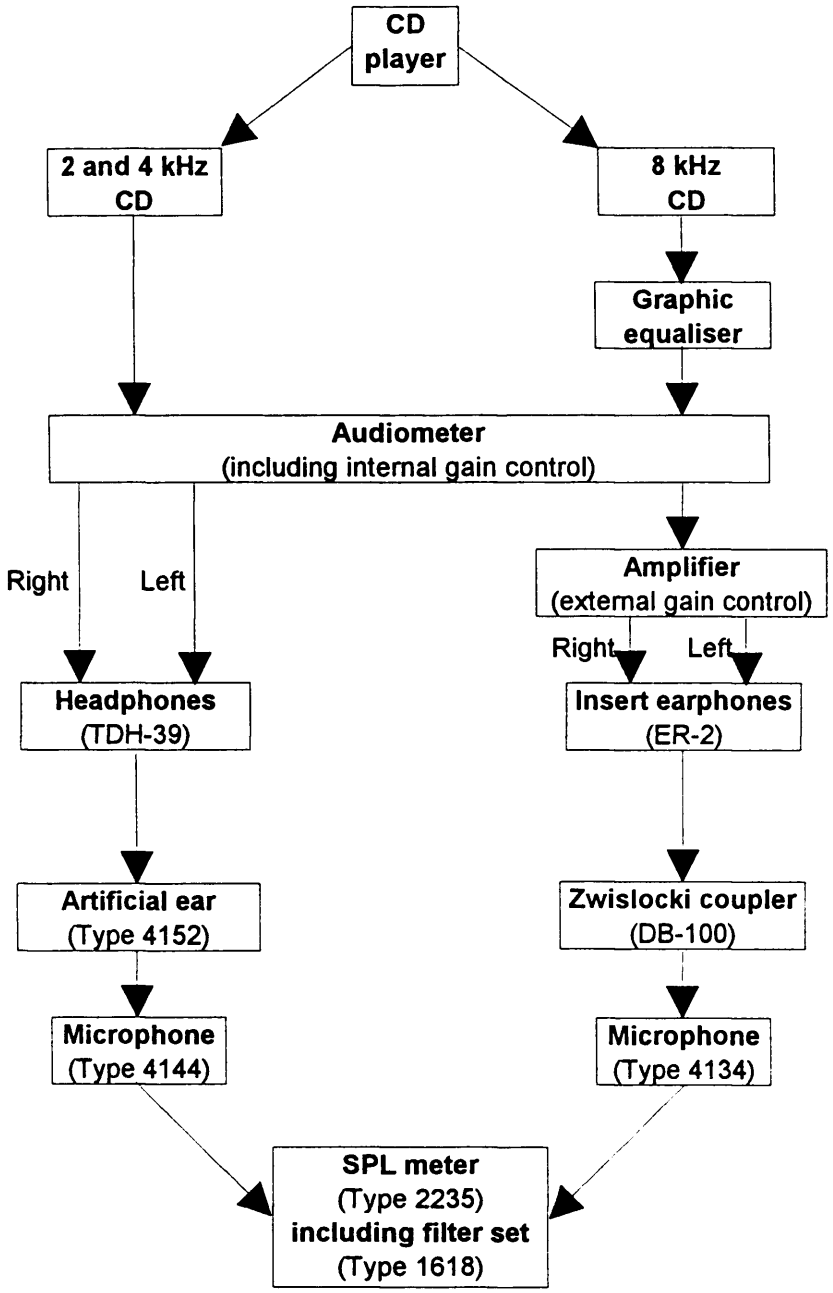


Fig. 2.4.1. Experimental set-up of equipment for frequency resolution measurements and calibration. See appendix 6.2.2. and 6.2.3. for exact specifications of test and calibration equipment respectively.

effects) of the audiometer and headphones on masker side-bands (i.e. with change in frequency response characteristics; the balance of noise levels to either side of notch) was checked by comparison with direct electrical signal analysis.

Acoustical checks for 8 kHz notched noise were made with the CD signal mixed through a graphic equaliser before the audiometer (with interface amplifier) and using Etymotic insert earphones (coupled to signal analyser via Zwislocki coupler, microphone and SPL meter; again see Figure 2.4.1.). The parameters checked for 2 and 4 kHz were similarly checked for 8 kHz. Furthermore, the graphic equaliser was used to ensure a flat output over the range of frequencies from 125 Hz - 16 kHz. To achieve a flat output: white noise at 0 dBVrms produced using the signal analyser as a source, was passed through the test / calibration equipment and back to the signal analyser; enabling adjustment of the graphic equaliser bands to flatten the frequency-response to within 3 dB over 125 Hz - 16 kHz.

2.4.2. RECORDING PARAMETERS

For each of the centre frequencies 2, 4 and 8 kHz there were four CD tracks, each track being approximately 3.5 minutes long. On each track was recorded a continuous noise. The noise consisted of two bands, each 0.4-times the centre frequency in width and positioned symmetrically to either side of that centre frequency. The four tracks for each centre frequency differed in terms of the separation of the two noise side-bands. Separation of the side-bands produced a notch, symmetrical with respect to the centre frequency. The width of each notch - i.e. the frequency separation between the leading edge of one of the side-bands and the centre frequency - was expressed as a fraction of the centre frequency. The notch widths of the noise recorded on the four tracks for each centre frequency were: 0 (un-notched), 0.1, 0.2 and 0.3. The total notch width to

either side of the centre frequency was therefore double the fraction of separation.

The level of the noise was set to 70 +/-2 dBSPL overall for all tracks and a spectrum level of 53 +/-3 dBSPL/Hz. The level of masking was determined by a compromise: too high and the masking would be uncomfortable for 10-15 minutes of testing and too low would be insufficient to raise the masked threshold above absolute threshold for the widest notched-noise.

2.4.3. IMPLEMENTATION OF TECHNIQUE

Headphones were used to assess 2 and 4 kHz, whilst 8 kHz was assessed using insert earphones. The pure tone threshold for the centre frequency in question was established +/-1 dB using a further modification to the method of limits (detailed below). The subject was asked to: "try to ignore the background noise and respond to the tone as before". The un-notched CD track was then played to the same ear, with the centre frequency tone first presented at either 55 or 60 dBHL and threshold values found for the pure tone as before. Threshold values were similarly obtained for the three tracks with the notched noise (in the order: 0.3, 0.1 and then 0.2). Finally, the centre frequency pure tone threshold was reassessed without the background noise. If the first and last absolute thresholds were not within 5 dB of each other, the results for that test session would be excluded from the study.

Establishment of a threshold to 1 dB resolution:

1. First establish a threshold to 5 dB resolution (see methods 2.3.4.).
2. Starting at 1 dB above threshold, presentations descended in 1 dB steps until a missed response, and then starting 1 dB lower, ascended in 1 dB steps until a positive response.

3. The process of descending and ascending presentations was repeated, starting at 2 dB above the level of the lowest positive response, until it was possible to assign a threshold as described below.
4. The lowest level to first yield two consecutive positive responses was recorded as a threshold value for that frequency.
5. A minimum of 6 threshold estimates were recorded for each set of conditions.

2.4.4. INTERPRETATION

Data obtained on the ability to resolve differences in frequency - at each test centre frequency - may be interpreted in two ways. Firstly, the difference between thresholds for the two extremes of no notch and the widest notch, can be calculated for immediate comparison (Moore et al., 1985). Secondly, a more thorough evaluation of the thresholds for all tracks can be performed by fitting the data to a simple computer model (Moore & Glasberg, 1983; Glasberg & Moore, 1990) for derivation of: rounded exponential (p); K; and, ERB (see introduction 1.8.6.). For symmetrical filter shapes near the tip of the tuning curve, the ro-ex(p) expression for a 'rounded top exponential' is used, where:

$$W(g) = (1 + p.g) \exp.(- p.g)$$

When: W is an intensity function describing filter shape; g is the deviation from the centre frequency divided by the centre frequency ($f - f_0 / f_0$); and, p is a parameter determining filter sharpness. The parameter p is calculated by fitting the integral of the equation to the data relating threshold to notch width. The equation appears to be adequate for characterising the shape of auditory filter to within 20-30 dB of the tip, where the pass-band can be assumed to be symmetrical. (Patterson et al., 1982.)

2.5. OTO-ACOUSTIC EMISSIONS

Oto-acoustic emissions are an objective physiological measurement used to establish the state of electromechanical amplification within the cochlea.

2.5.1. CALIBRATION

Calibration of the ILO systems was performed acoustically and electrically with reference to internal and external standards. The intensity and frequency of the output of transducer A and transducer B, and the input of the microphone were tested independently (2 probe DP system). The electronic hardware and probe were also assessed independently as recommended by Otodynamics Ltd. (D.T. Kemp *pers. comm.*, 7 March 1996). External reference checks were performed at the start and end of the study. However due to ease of execution, internal self-checks were performed at the start of each semester and whenever the equipment was moved.

The peripheral analogue box was tested independently of the probe, using ILO92 / 88 hardware test programme (with calibration plugs in probe sockets). External reference 'engineer testing' within the 'Test92' software comprised: a 1 kHz 2.0 V peak-peak sine wave (0.707 RMS) output independently at both A and B stimulus BNC ports on the rear of the analogue box which was confirmed by oscilloscope ('analogue box calibration'); and similarly, stimulus DAC output frequency-response (at 0 V adjustment, for sine waves of frequencies 0.7, 1, 2, 4, 6 and 8 kHz) was confirmed by signal analyser ('DAC output'). Internal self-checking comprised: user system checks with a diagnostic 'functional auto-test and report'.

The detachable probe was tested while connected to the analogue box. External reference testing comprised checking the transducers (over

ranges representative of DPOAE testing) and microphone (over ranges representative of SOAE testing), separately and using a 2 cm^3 coupling (with the ILO probe face flush to the opening in the centre plate of the 2 cm^3 coupler). The transducers were checked over 35 - 70 dBSPL in 5 dB increments, separately for 0.7, 1, 2, 4, 6 and 8 kHz (measured using a signal analyser to enable separation of signals from A and B when presented simultaneously) and running ILO92 DPOAE 'growth rate' tests at the aforementioned frequencies. The microphone was checked over 5 - 40 dBSPL in 5 dB increments, separately for 500 Hz - 8 kHz in octave increments (produced using a signal generator and Etymotic insert earphone*) and running the ILO92 'spectrum analyser' for 100 +/-5 averages with rejection set at maximum (20 mPa). Internal self-checking comprised: ILO92 distortion product analyser probe calibration, using the standard adult B-type probe test (checking: 1.0 cm^3 volume, by cavity SPL; and 'ear canal' frequency-response curve, to 70 dBSPL at 250 Hz); and, ILO92 distortion product system distortion, by running the DPOAE tests using the standard adult B-type probe in a 1.0 cm^3 cavity.

2.5.2. RECORDING PARAMETERS

SOAEs using the 'spectrum analyser' facility of the ILO92 software

The microphone sequentially records in 80 ms segments and calculates the frequency dependent intensity average for both SOAEs (if present) and system noise floor. Following Fourier analysis the result was presented as a discrete frequency spectrum (from 0 to 12 kHz) in 12.5 Hz +/-0.5 segments of varying intensity (from -16 to 90 dBSPL +/-0.35).

---

* The signal generator was used to drive an Etymotic insert earphone which was attached to the nipple of a 2 cm^3 coupler (via an additional 20 mm of tubing to account for the absence of an eartip). The signal generator was then calibrated with reference to a precision SPL meter and microphone traceable to NPL. The ILO probe was then positioned in place of the microphone and calibrated with reference to the signal generator.

CSSOAEs using the 'synchronised spontaneous search' facility of the ILO88 software

Click stimuli were presented at 80 dBpeSPL +/-4 (using a fixed voltage), of 5 kHz bandwidth and 80 μs duration, repeated every 80 ms. The microphone sequentially recorded in 80 ms segments. Following each click the first 20 ms was excluded enabling a time average of CSSOAEs (if present) to be measured, thereby reducing the system noise floor that was out of phase with the stimulus. Following Fourier analysis the result was presented as a discrete frequency spectrum (from 0 to 6.3 kHz) in 12.5 Hz +/-0.5 segments of varying intensity (from -45 to 35 dBSPL +/-0.1).

DPOAEs using the 'DP-gram' and 'Growth rate' facilities of the ILO92 software

Two tones (f_1 & f_2) different in frequency by a fixed ratio (1.22 +/-0.01) were presented simultaneously for 1.28 seconds, with a pause between presentations (the duration of which was determined by computer processing time). During each presentation of the stimulus tones the microphone recorded the intensity (from -30 to 50 dBSPL +/-0.1) within the distortion product window (centred at $2f_1$-f_2 with 5 points to either side, each point representing the SPL for a 12.5 Hz +/-0.1 wide band*), continuously during stimulation (representing 16 sub-averages each of 80 ms duration, for each tone pair presentation). The noise intensity was similarly recorded and presented as the first and second standard deviation, in order to clearly show any signal-to-noise overlap (where additional averaging may be necessary). If the noise exceeded a pre-set level (typically 3 mPa) for any section then that sub-average was not recorded. If the conditions were too noisy for all 16 sub-averages, then that data point was missed-out on the plot.

---

* i.e. a total of 11 points covering 136 Hz.

Distortion product audiograms

The intensity of the two tones was kept constant (both at 70.00 +/- 5 dBSPL) and the frequency was varied (f_2 = 700 Hz to 6 kHz +/-12.5 Hz, with 8 frequency points per octave).

Distortion product growth rates / input-output functions

The frequency of the two tones was kept constant (f_2 at 2, 4, 6 or 8 kHz +/-12.5 Hz) and the intensity was varied (from 70 to 35 dBSPL, in steps of 3 dB +/-1).

2.5.3. ASSUMPTIONS & LIMITATIONS

Of particular importance is the use of cues for artefact rejection. OAEs must be distinguished from the: stimulus sound; acoustic response of the middle ear; other patient noise; and environmental noise. To improve accuracy it is possible to check: non-linearity; latency; noise evaluation; and reproducibility of testing. To improve precision it is possible to use: fit stability; noise rejection threshold; number of averages; and response to noise ratio. Of especial value is signal enhancement by synchronous averaging and spectral analysis; enabling decreases in out of phase noise, different frequency noise and transient noise.

2.5.4. IMPLEMENTATION OF TECHNIQUE

Probe fit

Prior otoscopy provided guidance for placement of the probe. For hygiene and to provide a comfortable seal with the ear canal, an ear plug (with the centre 'punched-out') was manually moulded around the neck of the probe tip (as a collar) and rolled tightly in the fingers to compress the foam before sliding the probe tip into the ear canal (with a slight rotation of 30-60°). The body of the probe casing was then manually held in place while the foam expanded to provide a sealed fit between probe and canal.

During the fitting process the 'checkfit' program was left running and used as a guide to effective placement. The 'checkfit' program lasts 2-3 seconds per cycle, with each presentation comprising one 1.3 second 250 Hz tone followed by 16 click-evoked sub-averages (each sub-average comprising 4 clicks of 80 μs duration and 20 ms separation). The response to the clicks was presented as a spectral overlay from the two transducers (from 0 to 6 kHz & 20 to 60 dBSPL) and the cavity SPL in response to the tone enabled an estimation of the ear canal volume (from 1 to 4 cm^3). Useful cues for assessing goodness of probe fit included: a stable volume (+/- 0.1 cm^3 over three successive measures) between probe tip and eardrum; no ringing of the stimulus in the ear canal; a flat frequency-response showing no indication of standing waves (caused by resonance due to distance between probe tip and eardrum); and no separation in amplitude of the response from the two tones (caused by obstruction due to the two transducers not both pointing in direct alignment with a clear line to the eardrum).

Signal to noise ratio

The signal to noise ratio varied between tests, depending on differences in subject movement and background noise. The signal to noise ratio was moderated by the number of averages recorded and the rejection threshold. Typical settings for the 3 types of OAE test were as follows: 100-300 averages with rejection set at 3-4 mPa for SOAEs; 260 averages with rejection set at 1.2-5.2 mPa for CSSOAEs; and 1-4 averages at each point with rejection set at 3-4 mPa for DPOAEs. When considering the three different types of OAE methods employed: only discrete frequency peaks occurring 6 dB or more above the noise floor (+/- 50 Hz) were considered to be SOAEs or CSSOAEs; and $2f_1$-f_2 emissions occurring above two standard deviations of the noise floor were considered to be DPOAEs.

2.6. CRITERIA FOR ASSESSING THE OUTCOME OF THE STUDY

Ultimately, the main objective of the project was to investigate how cochlear performance changes with aminoglycoside doses used clinically. The research must first establish the degree of variation within each type of test employed. Such control data could then be used for comparison with any other group of subjects receiving potentially ototoxic agents. Finally, differences between the control and patient data may be used to answer pre-specified hypotheses concerning the effects of the patient condition and treatment.

2.6.1. STABILITY OF TECHNIQUES AND PRIMARY HYPOTHESIS

Stability was assessed for all tests on control subjects by calculating three main types of variability: inter-aural (between ears); inter-individual (between people), and intra-individual (over time / test-retest). In addition, the correlation with age in control subjects was investigated for all tests. Comparison of control subjects with aminoglycoside exposed CF patients enabled the primary hypothesis of the study to be investigated. The hypothesis is that the progression of sub-clinical deficit detection by tests would be most pronounced in OAEs, and /or frequency resolution, before PTA (for rationale see introduction 1.8.12.).

2.6.2. DATA ANALYSIS

Initially, each test type was considered in turn to assess the variability of the control data. Then the above hypothesis was inverted into a null hypothesis for disproval. Pre-experimental power calculations were not performed due to an oversight. Instead, as many patients as possible were recruited and tested within the time available. Nevertheless, post-experimental power calculations were made, to ensure significant

differences were not missed due to insufficient data. All power calculations were made using 0.8 probability of detecting a true difference (80 % true positive) and accepting a 0.05 probability of a false difference (5 % false positive), whilst assuming homogeneous variance and sample sizes. In order to test hypotheses, appropriate statistical comparisons were made (always after checking for normality of distribution and homogeneity of variance) using Microsoft Excel and Minitab software packages. However, all values were presented as mean +/-SD (even though the non-normality of individual groups may have dictated otherwise) in order to facilitate easier comparisons (both within the study and with published literature).

Test-retest variability was assessed by partitioning the variance between am-to-pm (for one week) and week-to-week (for one year), for all tests where possible (due to poor subject compliance psychophysical tests included month-to-month instead of week-to-week measures). The effects of age (in control subjects) and dose (in patients*) were assessed by scatter plot and regression analysis, for all tests where necessary (DP-gram results could be inferred from DP input-output function results). Finally, control subject and patient groups were directly compared using appropriate two-tailed statistical tests - determined as aforementioned by the distribution of the data (by observing scatter plot and calculating skew and kurtosis) within each group and equality of variance between groups (by F-test) - again for all tests (partitioned by time, age and / or dose if previously found to be necessary). The statistical tests employed were: Type II (pooled) T tests for normally distributed unequal group sizes with homogeneous variance; Type III (two-sample / student's) T tests for normally distributed unequal group sizes with heterogeneous variance; and, Mann-Whitney U (Wilcoxon ranked sum) tests for non-normally distributed groups. In addition, categorical data was evaluated using Chi-squared (χ^2) tests when expected group sizes were

---

* Patients with sensorineural loss did not contribute to regression analyses.

all >5, or Fisher's Exact tests whenever expected group sizes were <5. Finally, where subjective designation of a result occurred (e.g. designation of DPOAE input-output function shape), it was of paramount importance to be consistent across the two groups and not have any pre-conceived ideas that may bias the outcome.* The exact statistical treatment used in a specific measurement is indicated underneath the data in the relevant tables or in the text.

2.6.3. JUSTIFICATION OF CHOSEN ANALYTICAL APPROACHES

As always, there were a number of different approaches that could be chosen for the analysis of the data. There are advantages and disadvantages associated with each approach and the best analytical method is a matter of opinion. The following discussion aims to clarify the reasoning behind the analytical methods adopted.

The same exclusion criteria were applied equally to control subjects and patients. Patients who had received no aminoglycoside therapy were included in dose-response analyses, but were excluded from comparisons of patients with control subjects. Initially, all individuals with sensorineural loss** were also excluded for comparison of control and patient groups in order to test for sub-clinical differences. However, patients with bilateral sensorineural loss (which could be considered indicative of ototoxicity), were re-included in separate analyses in order to test for gross differences between control and patient groups as a whole. Patients with unilateral sensorineural loss were not included as there was also a similar proportion of excluded control subjects with unilateral sensorineural loss*** (that was

---

* Also, all measurements interfere with and change the processes that they aim to measure; so one should always attempt to ensure no site, equipment or operator bias.

** From here on, sensorineural loss refers to cases confirmed by standard PTA (for criteria see methods 2.2.1.).

*** All individuals with unilateral sensorineural loss had both ears excluded.

of uncertain origin). In contrast, both patients and control subjects with unilateral sensorineural loss could have been included, but the control subjects would not have been followed up as thoroughly and so would be underrepresented in comparison with patients. Nevertheless, the lack of any control subjects with bilateral sensorineural loss could be considered an artificial reflection of the PTA screening criteria. Thus it is difficult to achieve a satisfactory comparison involving patients with sensorineural loss.

There were a number of considerations involving the possible division of both control and patient groups before comparison, including ear, age and gender asymmetries. Firstly, for comparisons of control and patient groups the use of principle components analysis (to partition the variance between contributing parameters) was considered, but rejected due to the lack of complete data sets for all tests. Secondly, for all tests, right and left ears were analysed separately, as the ears of one individual cannot be considered independent measures (and pooled). In addition, not all tests were completed bilaterally, so there will be varying contributions from patients with potential sub-clinical deficits depending on whether results from one or both ears were available. Thirdly, any age-related test effects were investigated by scatter plots and correlation, with the intention of separating patient and control subjects into juvenile and adult groups prior to subsequent analysis if necessary.* Finally, the reported higher prevalence of SOAEs in females compared with males (Whitehead et al., 1989) warranted investigation for any gender asymmetries in both SOAEs and CSSOAEs (although approximate sex-matching across groups removes the need to separate analyses by gender).

Frequency resolution was only expressed as the difference (in dB) between widest notch and no-notch thresholds, due to a lack of time to

---

* Even though control and patient groups were approximately age-matched and both had continuous age ranges, recruitment did fall into adult and juvenile groups and so any systematic differences should be assessed.

develop the computer program. It is anticipated that the computer program will become available in the future, enabling a more complete analysis.*

For each DPOAE input-output function measurement it was possible to record -10 dBSPL iso-criterion, slope and shape (see results 3.6.). The use of linear calibration (to predict X from Y) was considered, but rejected due to the complexity of fitting lines to non-linear shapes. Furthermore, DPOAE input-output functions had a restricted range of stimulation from 35-70 dBSPL. Some input-output functions will continue down past 35 dBSPL and would intercept the -10 dBSPL response level at an unknown lower stimulus amplitude. Other input-output functions will not be evident below 70 dBSPL (falling within the 'none' category of shape) and would intercept the -10 dBSPL response level at an unknown higher stimulus amplitude. Input-output functions with -10 dBSPL iso-criteria at unknown stimulus levels below and above the test limits could be assigned minimum and maximum values (i.e. 35 and 70 dBSPL respectively). Alternatively, iso-criteria below and above the test limits could be excluded. Either attributing minimum and maximum values or excluding these input-output functions would bias the data groups. However, the shape of the input-output function could be used to extrapolate to lower stimulus iso-criteria. The 'safe' limit for such extrapolation could then be judged by the normality of the resulting data distribution and additionally an evidence-based minimum value could be attributed. When using identical extrapolation criteria, the distribution of scatter plots revealed the 'safe' lower limit of extrapolation to be 20 dBSPL in stimulus level. Therefore, for lower stimulus iso-criteria it was decided to extrapolate and also designate a minimum value (20 dBSPL). On the other hand, higher stimulus iso-criteria were excluded as there was

---

* For frequency resolution measurements, fitting a curved line to four point measurements has been discussed, other authors have used between 5 and 19 points (Moore & Glasberg, 1983; Stone et al., 1992), and 5 points appears to optimise variability (Leeuw & Dreschler, 1994).

no such evidence on which to base a value. In addition, there was concern that attributing a maximum value may disrupt the normality of the data distribution. Incidentally, the similar (or even higher) proportions of 'none' input-output functions in control groups at all frequencies indicates that designation of an arbitrary maximum value (i.e. 70 dBSPL) would not result in patients performing more poorly.

A number of criteria should be considered when characterising SOAEs: peaks must be within a predetermined width (e.g. 25 Hz) at a fixed level down from the tip of the peak (e.g. 6 dB); peak height must be equal to, or greater than a fixed level (e.g. 6 dB; equivalent to 2 SD of mean overall spectrum) above mean surrounding 'noise' (e.g. +/-50 Hz); 'noise' must also be continuous to either side of a peak (e.g. for 25+ Hz of a 50 Hz window), in order to distinguish juxtaposed peaks. Even so, some SOAEs will fail to be detected in consecutive spectral analyses because either SOAE and / or noise fluctuate in level.* Thus, the designation of SOAEs is a complex problem. For both SOAEs and CSSOAEs, the use of control charts to identify peaks (by separating outliers from a fluctuating noise floor) was considered, but rejected due to the complexity of examining sequential frequency sections. The alternatives were to apply a template of mean noise and designate any peak above +2 SD as a SOAE, or to use a fixed level amplitude for all frequencies (a 'flat-line cut-off') above which any peak was designated as a SOAE. Both methods require the contribution of noise to be approximately equal between control and patient groups, and thus it is necessary to exclude data below 1 kHz due to the greater susceptibility of lower frequencies to noise interference. As the intention is to directly compare groups, then relative rather than absolute measurements are important. Therefore, the actual method of SOAE designation is of low

* Although it is possible that SOAEs could 'float' slightly with background noise before being swamped.

importance as long as all groups are treated equally in this respect. Finally, the fixed level amplitude method was implemented. An absolute amplitude of 0 dBSPL was considered conservative, less labour intensive, allowed easier interpretation across frequencies, enabled direct comparison with the CSSOAE method, and also represented the extreme lower intensity limit of calibration.

RESULTS

First of all it was necessary to consider which data were to be included / excluded. Secondly, the exposure of CF patients to intravenous aminoglycosides was evaluated. Then each test type was analysed separately (in the order: high frequency pure tones; frequency resolution; DP-grams; DPOAE input-output functions; SOAEs; and, CSSOAEs), considering: test-retest variability; effect of age; dose-response relationship; and, comparison of control with patient groups. Finally, the relationship between different tests was evaluated - where possible - for control subjects.

3.1. INCLUSION / EXCLUSION STATISTICS

3.1.1. TEST LOCATIONS

Free-field measurements for background noise were always less than: 30 dBSPL at 250 Hz; and, 10 dBSPL at all other frequencies. Acceptable ear canal attenuated measurements for background noise were always less than: 10 dBSPL below 1 kHz; and, 0 dBSPL above 1 kHz.

3.1.2. CALIBRATION

When comparing calibrations from the start and end of the project: intensities were found to be within 3 dB (typically less than 5 %); frequencies within 5 Hz (typically less than 1 %); and, pressures within 40 daPa (typically less than 15 %). It was not always possible to assess the functioning of the ILO92 transducers at 6 and 8 kHz due to distortion - caused by differences in checkfit - which prevented stabilisation of the stimulus levels. In addition, assessing the function of the ILO92 microphone at frequencies below 1 kHz was more susceptible to influence by background noise. The calibration enabled calculation of

correction factors for the conversion of all test results into dBSPL, with the exception of standard pure tone audiometry (which was expressed in dBHL).

3.1.3. RECRUITMENT OF SUBJECTS

Overall, only approximately half of all approached patients (both juvenile and adult) agreed to be tested, approximately half of those who agreed to be tested failed to attend for testing, and approximately half of those who attended for testing failed to re-attend to complete all the tests. Finally, 84 patients (40 male and 44 female) were recruited and tested, ranging from 5 to 37 years of age (mean+/-SD = 20.2+/-8.1 years). The lower age limit of 5 years was determined by ability to perform psychophysical testing; whereas, the upper age limit was determined by the confounding effects of presbyacusis, transplant care and death (usually around middle-age). Patients were recruited from four hospitals: Leicester Royal Infirmary (n = 19); Northampton General (n = 10); Peterborough District (n = 12); and, Birmingham Heartlands (n = 43). Given that there were no known differences in therapeutic policies, patients from different sites were merged into one group. Diagnosis of cystic fibrosis was confirmed genetically in 48 patients (57 %). For a further 18 patients one or both of their alleles could not be identified; as either none of the commonly screened alleles were present*, or the test was designated a scientific failure. The remaining 18 patients had not undergone CF genotyping.

Juvenile control subjects were necessarily recruited by teachers or parents, and - similar to patients - approximately half of those who agreed to be tested failed to attend for testing, and approximately half of those who attended for testing failed to re-attend to complete all the tests. In contrast, approximately 80 % of potential adult control subjects agreed to be tested when approached, and 100% of those who agreed

---

* ΔF508, G551D, R553X, G542X and 621 + 1G>T account for 83 % of CF genotypes in the UK (regional NHS medical genetics department).

to be tested also attended for testing, although again approximately half of those who attended for testing failed to re-attend to complete all the tests. Control subjects were as closely matched as possible in sex and age to the patient group (as the study neared completion, recruitment and testing focused only on underrepresented control subgroups). Thus, 108 control subjects (54 male and 54 female) were recruited and tested, ranging from 6 to 36 years of age (mean+/-SD = 20.5+/-6.5 years). Control volunteers were recruited from Leicester Grammar School (n = 57); patient's siblings (n = 2); and, Leicester University (n = 49). Assuming no difference in genetic nor environmental conditioning on the auditory system, individuals were merged into one group.

3.1.4. TYMPANOMETRY

Due to failure to comply with tympanometry inclusion criteria, 3 patients (1 of which had grommets) and 4 control subjects (1 of which had no tympanometry) were completely excluded. A single ear was excluded due to tympanometry for 6 patients (4 left and 2 right ears) and 7 control subjects (3 left and 4 right ears). Tympanometry also varied slightly from test-to-test within the same individual when tests occurred on different dates.

3.1.5. STANDARD PURE TONE AUDIOMETRY

Due to failure to comply with pure tone audiometry inclusion criteria, 11 patients (6 of which had no audiogram, and 1 of which had a problem in the supratentorial plane) and 6 control subjects (1 of which had no audiogram) were excluded. In addition to those excluded by PTA above, 9 patients had sensorineural loss (6 bilateral and 3 unilateral; see Figures 3.1.5.1., 3.1.5.2. and 3.1.5.3.), and 4 control subjects had sensorineural loss (all unilateral).

Figures 3.1.5.1.A. and B. show PTA for 3 patients with severe bilateral losses: L17 was a 16 year old female, having received 231 days of aminoglycoside treatment and a history of epilepsy; H25 was a 29 year old male, having received 105 days of aminoglycoside treatment and a history of renal sarcoidosis and reported tinnitus, vertigo, and otalgia associated with past treatments; and H32 was a 27 year old male, having received 535 days of aminoglycoside treatment with an otherwise unremarkable history.

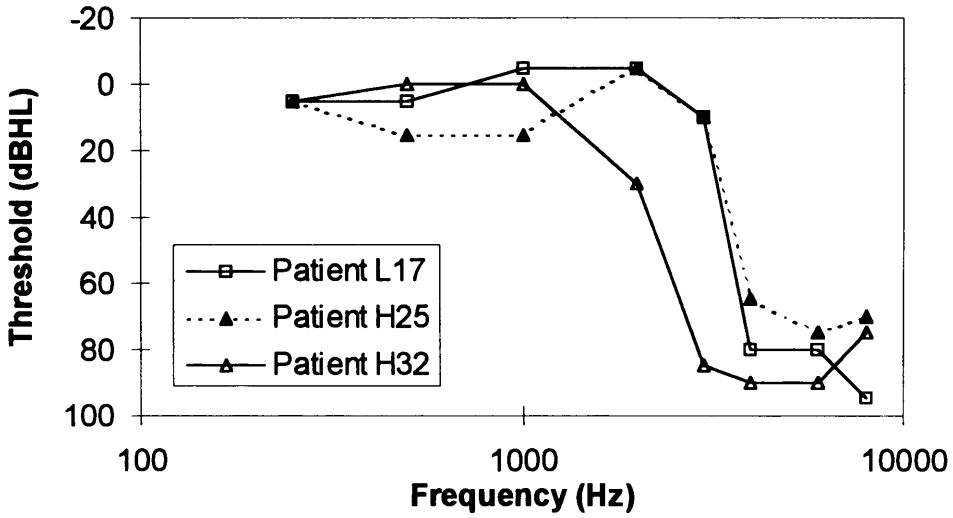


Fig. 3.1.5.1.A. PTA of patients with severe bilateral sensorineural loss: Left ear

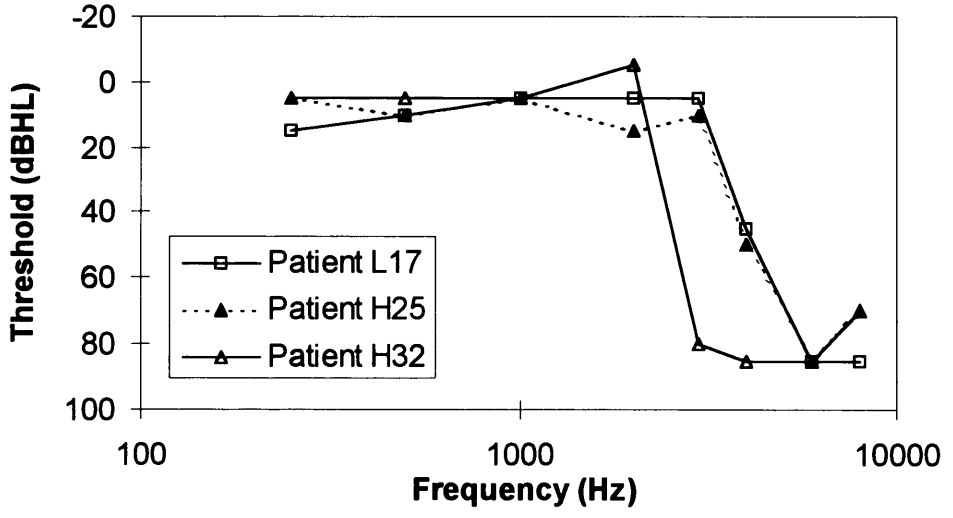


Fig. 3.1.5.1.B. PTA of patients with severe bilateral sensorineural loss: Right ear

Figures 3.1.5.2.A. and B. show PTA for 3 patients with moderate bilateral losses: L3 was a 23 year old female, having received 381 days of aminoglycoside treatment with an unremarkable history; H37 was a 28 year old male, having received 428 days of aminoglycoside treatment and reported severe nausea associated with past treatments; and H41 was a 36 year old male, having received 116 days of aminoglycoside treatment with an unremarkable history.

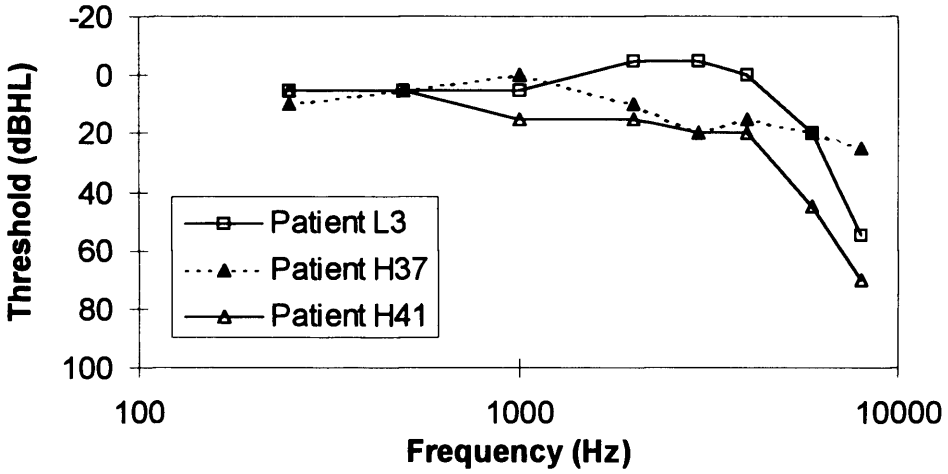


Fig. 3.1.5.2.A. PTA of patients with moderate bilateral sensorineural loss: Left ear

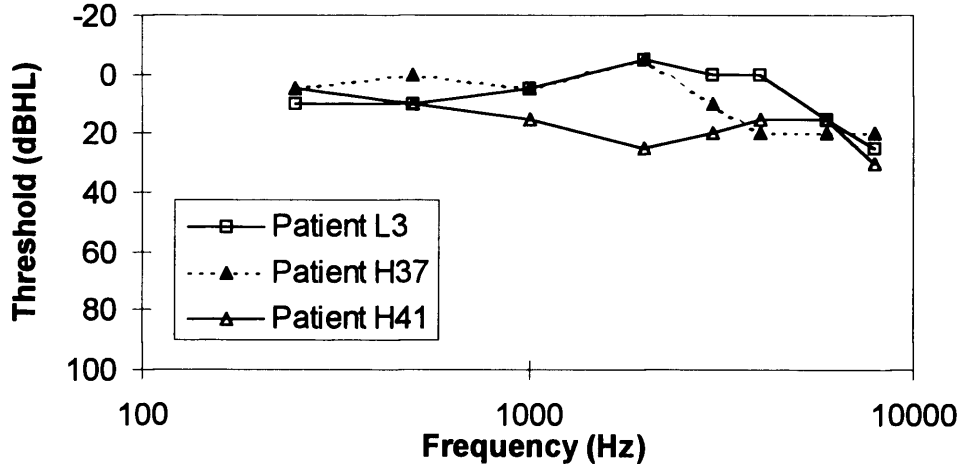


Fig. 3.1.5.2.B. PTA of patients with moderate bilateral sensorineural loss: Right ear

Figures 3.1.5.3.A. and B. show PTA for 3 patients with unilateral losses: H27 was a 27 year old female, having received 529 days of aminoglycoside treatment and reported tinnitus with an accidental high dose; H31 was a 19 year old female, having received 580 days of aminoglycoside treatment with an unremarkable history; and H33 was a 31 year old female, having received 113 days of aminoglycoside treatment and also reported tinnitus with an accidental high dose.

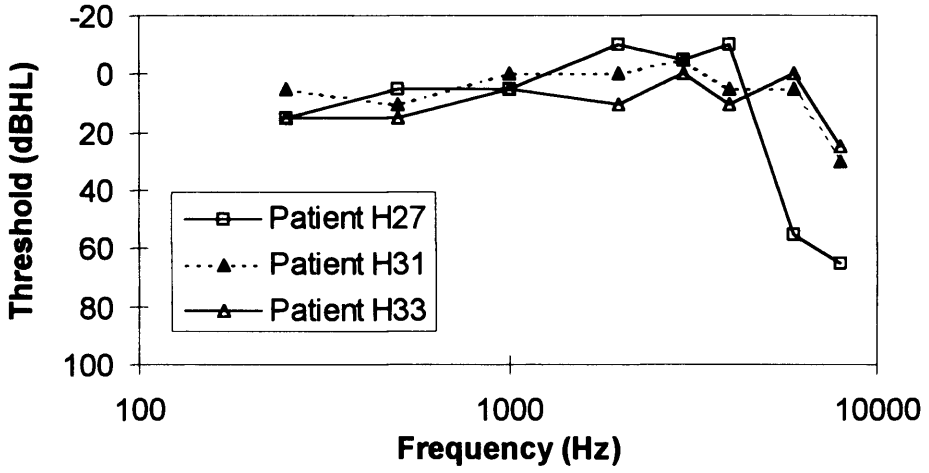


Fig. 3.1.5.3.A. PTA of patients with unilateral sensorineural loss: Left ear

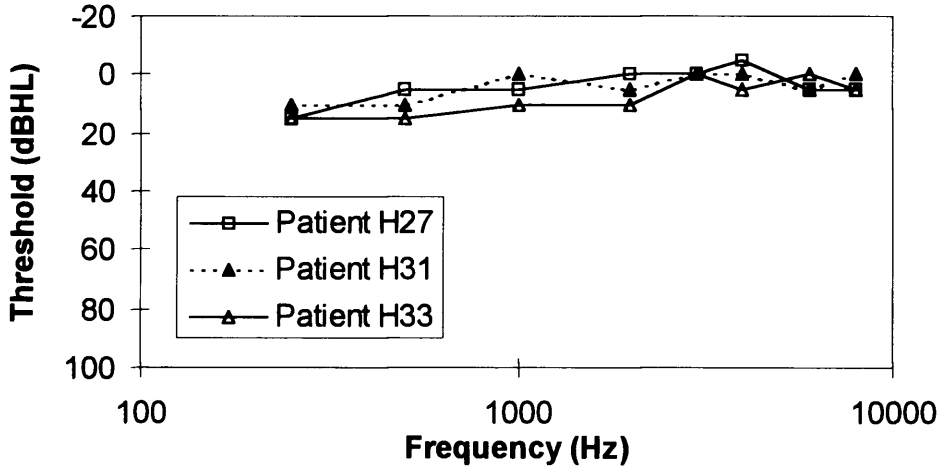


Fig. 3.1.5.3.B. PTA of patients with unilateral sensorineural loss: Right ear

Hence, 106 control subjects and 78 patients completed all screening tests. Of those, 8 control subjects and 8 patients failed inclusion criteria outright (χ^2 = 0.42, df = 1, p = 0.51). An additional 4 control subjects and 3 patients had unilateral sensorineural losses, and 6 patients had bilateral sensorineural losses. Therefore, 4 out of 98 remaining control subjects and 9 out of 70 remaining patients had clinical signs potentially indicative of ototoxicity (χ^2 = 4.41, df = 1, p = 0.036). (See Table 3.1..) Furthermore, separating paediatric from adult patients (into age categories of 5-19 and 20-37 years old) reveals sensorineural losses in 2 out of 36, and 7 out of 34 patients, respectively (Fisher's Exact, p = 0.60). However, 3 patients were excluded out of the juveniles and 5 patients were excluded out of the adults. Hence when including all screened patients, 18.0 % of adult patients and 5.1 % of paediatric patients had high frequency sensorineural loss (11.5 % overall).

Group	Recruited	Completed screening	Accepted / Rejected by screening	Additional unilateral sensorineural losses	Additional bilateral sensorineural losses
Controls	108	106	98 / 8	4	0
Patients	84	78	70 / 8	3	6

TABLE. 3.1. The number of individuals included and excluded for both control and patient groups.

3.2. AMINOGLYCOSIDE DOSAGE

3.2.1. TOTAL EXPOSURES

External aminoglycoside dosage was most readily represented - at time of testing (+/-1 day) - in terms of prior administration of a total number of: grams; days; and, courses. Generally, paediatric patients received gentamicin and adult patients received tobramycin (usually subsequent to treatment with gentamicin as a child), but there was considerable overlap. In addition, 10 of the patients had each received 1-3 courses of one or more of: amikacin; netilmicin; and, neomycin. The responses from these patients were not notably abnormal, and it is therefore assumed that any difference in toxicity between different aminoglycosides was less than the difference in dosage, absorption, distribution, metabolism and excretion. Hence, different aminoglycosides were merged to calculate overall exposure (in terms of external dosage).

The maximum total intravenous aminoglycoside exposures received by any patient were 343 g, 700 days, and 53 courses. One course was typically for two weeks at 0.7-1.7 mg/kg every 8 hours. Total dose was a minimum estimate based on judicious moderation encompassing all available sources of information on therapy (patient records and questioning; see methods 2.2.2.). Total dose also varied from test-to-test within individuals when tests occurred on different dates. At the time of testing, six patients (out of the 70 who successfully completed screening) had received no intravenous aminoglycoside therapy whatsoever.

3.2.2. EXTERNAL VERSUS INTERNAL MEASURES

Internal aminoglycoside dosage was measured by taking serum levels, usually at the third administered dose and following every change in external dose for each course of treatment. Serum levels accepted as optimum for therapy (by the consultants concerned) were trough (pre-dose) of <2.0 mg/l and peak (1 hour post-dose) of between 5-12 mg/l.

For all analyses of internal dosage, only gentamicin and tobramycin were included. For both tobramycin and gentamicin there was a varying correlation ($p < 0.1$-0.001) between external and internal dosage (although the correlation was better between individuals*, Fig. 3.2.2.1.; than, within an individual**, Fig. 3.2.2.2.). *** Ideally, total internal dose would have been quantified and used for all subsequent correlations, but serum level measurements were far too infrequent for this to be possible. As exposure depends on duration as well as level, it was considered reasonable to express total dose in terms of total days treatment. Total days treatment then represents a known duration when internal dose was aimed to be within a desired range. In support of this there was a good correlation ($r = 0.92$; $p < 0.001$) between total exposure in grams and days (Fig. 3.2.2.3.), suggesting that grams or days would be equivalent predictors of the total dose received.

---

* For odd numbered patients the first recorded peak serum level was used, and for even numbered patients the last recorded peak serum level was used. (All recruited patients for whom records were available were included.)

** For the patient with the most serum level data, the first recorded peak serum level was used for every recorded course of treatment. (This patient also had sensorineural loss.)

*** Figures 3.2.2.1-2. both show most doses to be in excess of the recommended 0.7-1.7 mg/kg, this is accounted for by clinicians moderating dose upwards to achieve the desired serum level (see methods 2.2.2. and discussion 4.3.4.).

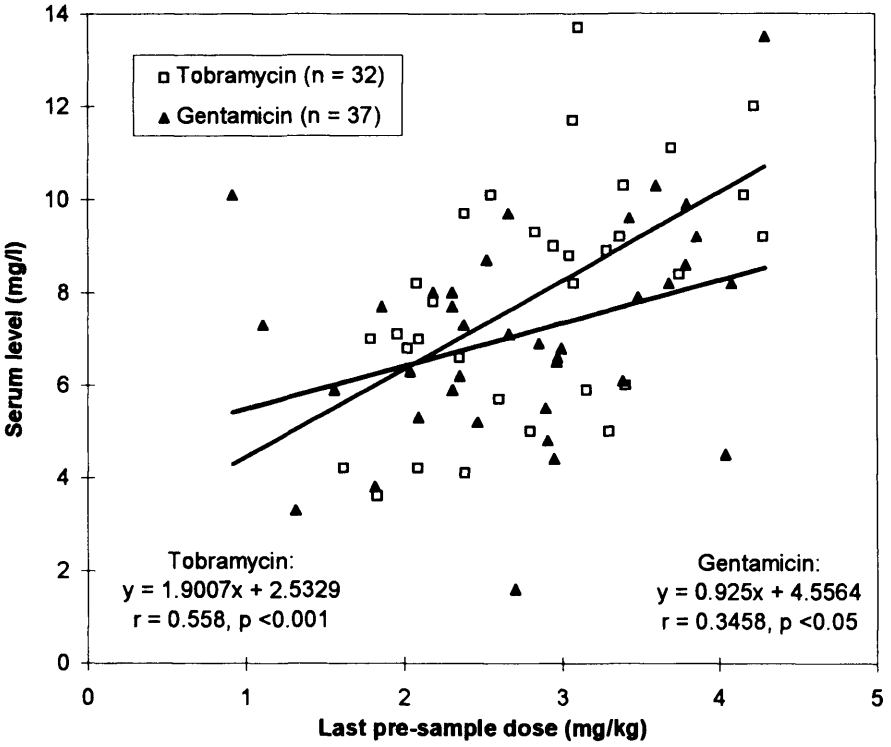


Fig. 3.2.2.1. Relationship between external and internal dose measures for different patients

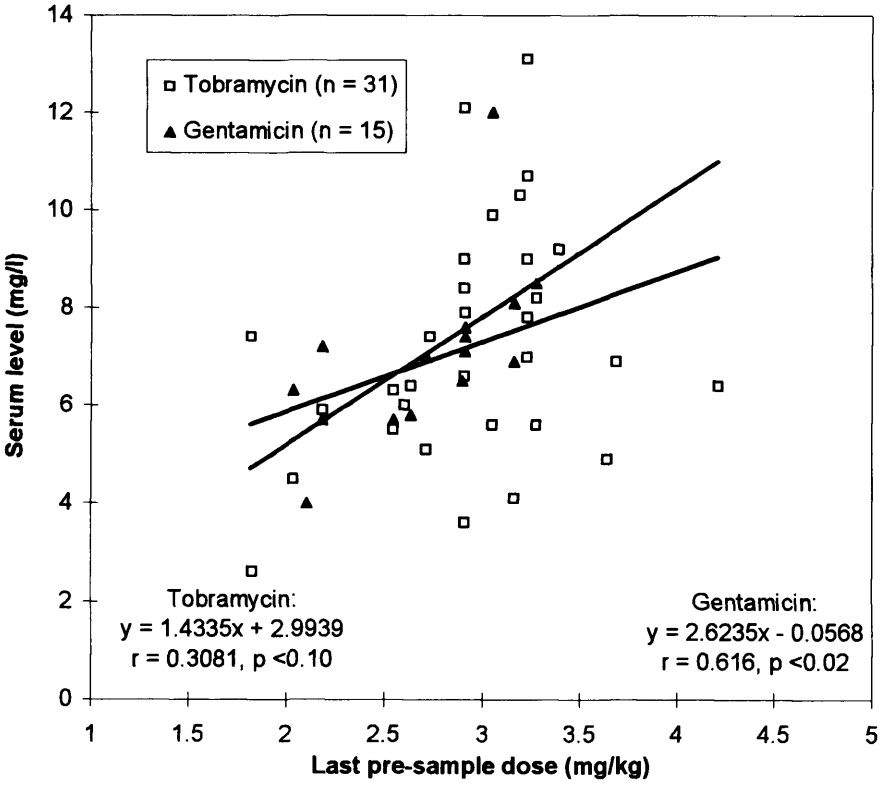


Fig. 3.2.2.2. Relationship between external and internal dose measures within one patient (H32)

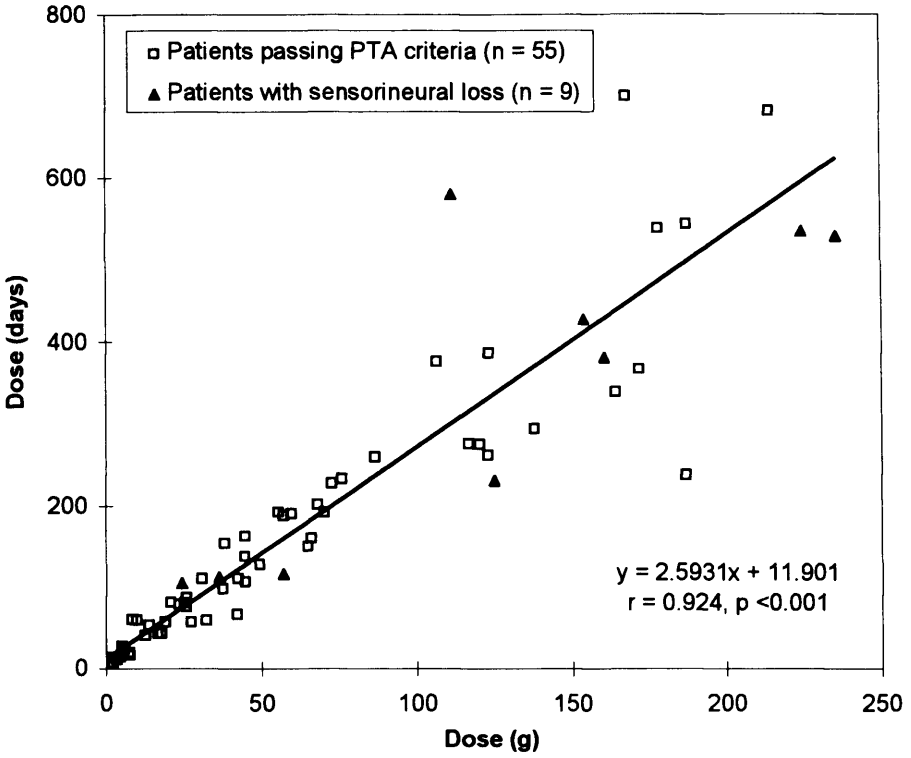


Fig. 3.2.2.3. Relationship between total dose measures of grams and days

17.4 % of all reported aminoglycoside courses were estimated (17.3 % gentamicin and 17.5 % tobramycin courses were completely interpolated; see methods 2.2.2.). Overall only 63.7 % of all potential serum levels were known (70.5 % gentamicin and 59.0 % tobramycin), assuming one measurement for each course and change of dose within each course. Of known serum levels: 7.6 % of peak levels were below 5 mg/l (6.6 % gentamicin; 8.4 % tobramycin) and 5.7 % were above 12 mg/l (4.0 % gentamicin; 7.2 % tobramycin); also, 4.2 % of known trough serum levels were 2 mg/l or more (1.7 % gentamicin; 6.2 % tobramycin). Therefore, a given number of days total treatment reflects a known period of time when peak serum levels can be assumed to be within the desired therapeutic range 86.7 % of the time* (89.3 % for gentamicin; 84.5 % for tobramycin). In all cases, serum levels appear to have been more carefully controlled with gentamicin than tobramycin.

---

* i.e. assuming data gaps to be randomly distributed; as peak serum levels would have been within the desired range on 86.7 % of treatment days.

3.2.3. PEAK EXPOSURES

Only known serum levels can, of course, be included. For all patients, maximum trough serum levels ranged from 0.5 to 5.7 mg/l (mean+/-SD = 2.0+/-1.0 mg/l, n = 61). Trough serum levels of 2 mg/l or more were recorded in 23 patients (once in 17; twice in 3; and, three times in 3 patients). However, trough serum levels only exceeded 3 mg/l in 9 patients. For all patients, maximum peak serum levels ranged from 5.2 to 20.0 mg/l (mean+/-SD = 10.9+/-2.8 mg/l, n = 61). Peak serum levels above 12 mg/l were recorded in 19 patients (once in 10; twice in 4; three times in 3; and, four times in 2 patients). Therefore, a total of 9 patients had exceeded the recommended (see methods 2.2.2.) maximum peak serum level on two or more occasions.

3.2.4. EFFECT OF EXPOSURE ON SENSORINEURAL LOSS

Patients with sensorineural loss were exposed to significantly more total days treatment than patients passing the PTA criteria (mean+/-SD (n) = 335.3+/-196.7 (9), and 153.9+/-163.6 days (61) respectively; Mann-Whitney U test, p = 0.006). However, patients with sensorineural loss were also significantly older than patients passing the PTA criteria (mean+/-SD (n) = 26.2+/-6.1 (9), and 19.1+/-8.0 years (61) respectively; Type II T test, p = 0.01).

3.2.5. EFFECT OF AGE ON EXPOSURE

The overall exposure of patients to aminoglycoside therapy was 177.2+/-177.5 days, when including those with zero exposure (mean+/-SD, n = 70); and 193.9+/-176.7 days, when excluding those with zero exposure (mean+/-SD, n = 64). There was also a significant trend (r = 0.37; p <0.01) for cumulative dose to increase with age (Fig. 3.2.5.).

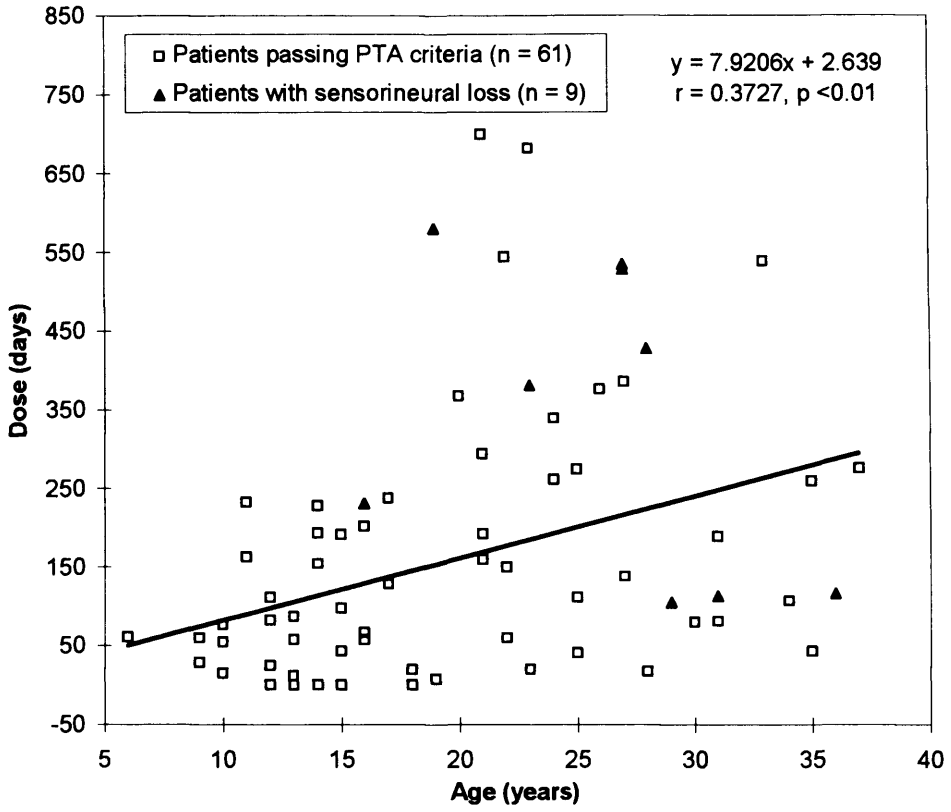


Fig. 3.2.5. Effect of age on total days dose

In later categorical comparisons, age and dose were each split into two approximately equal group sizes (which also conveniently coincided with the natural recruitment split between grammar school and university). Young patients (5-19 years) were exposed to 97.6+/-114.5 days therapy (mean+/-SD, n = 36), and old patients (20-37 years) were exposed to 261.6+/-194.2 days therapy (mean+/-SD, n = 34). Thus, young patients were exposed to significantly less aminoglycoside treatment (Mann-Whitney U test, $p < 0.001$). Conversely, low dose patients (0-99 days*) were 16.3+/-7.2 years old (mean+/-SD, n = 31), and high dose patients** (100-700 days) were 23.0+/-7.1 years old (mean+/-SD, n = 39). Thus, low dose patients were also significantly younger (Type II T test, $p < 0.001$).

---

* 100 days approximates to 8 courses.

** All of the patients with high frequency sensorineural loss were in the high dose category.

3.3. HIGH FREQUENCY PURE TONE AUDIOMETRY

3.3.1. TEST RE-TEST VARIABILITY

For high frequency pure tone thresholds (at 10, 12, 14 and 16 kHz), repeated measurements in one representative control individual were found to be approximately equally stable, irrespective of the time of testing (see Table 3.3.1.).

Frequency	Test interval	Left ear threshold mean +/-SD (dBSPL)	Right ear threshold mean +/-SD (dBSPL)
10 kHz	AM-PM	25.0 +/-3.3	11.5 +/-4.1
	Month-Month	24.6 +/-4.0	17.5 +/-6.6
12 kHz	AM-PM	32.5 +/-2.6	26.5 +/-2.4
	Month-Month	35.0 +/-3.7	28.3 +/-5.8
14 kHz	AM-PM	46.0 +/-3.2	45.5 +/-1.6
	Month-Month	46.7 +/-4.4	44.6 +/-5.4
16 kHz	AM-PM	59.0 +/-2.1	48.0 +/-4.8
	Month-Month	62.1 +/-4.0	54.2 +/-5.6

TABLE. 3.3.1. Test-retest variability of high frequency PTA (at 10, 12, 14 and 16 kHz): AM-PM over one week (n = 5 days AM and PM) and month-month over one year (n = 12 months).

3.3.2. EFFECT OF AGE

High frequency pure tone thresholds at 10, 12, 14 and 16 kHz were mostly found to correlate with age in control subjects ($p < 0.05$). Scatter plots and regression analyses are shown, for all four frequencies in Figures 3.3.2.1., 3.3.2.2., 3.3.2.3. and 3.3.2.4. respectively. The correlation was strongest ($p < 0.001$) for both ears at 16 kHz. In contrast, there were only two instances without a significant correlation ($p > 0.05$). The two exceptions occurred at 12 and 14 kHz, and for the left ear only. Hence, for subsequent comparisons of patients and control subjects it was deemed appropriate to partition groups according to age for all four frequencies.

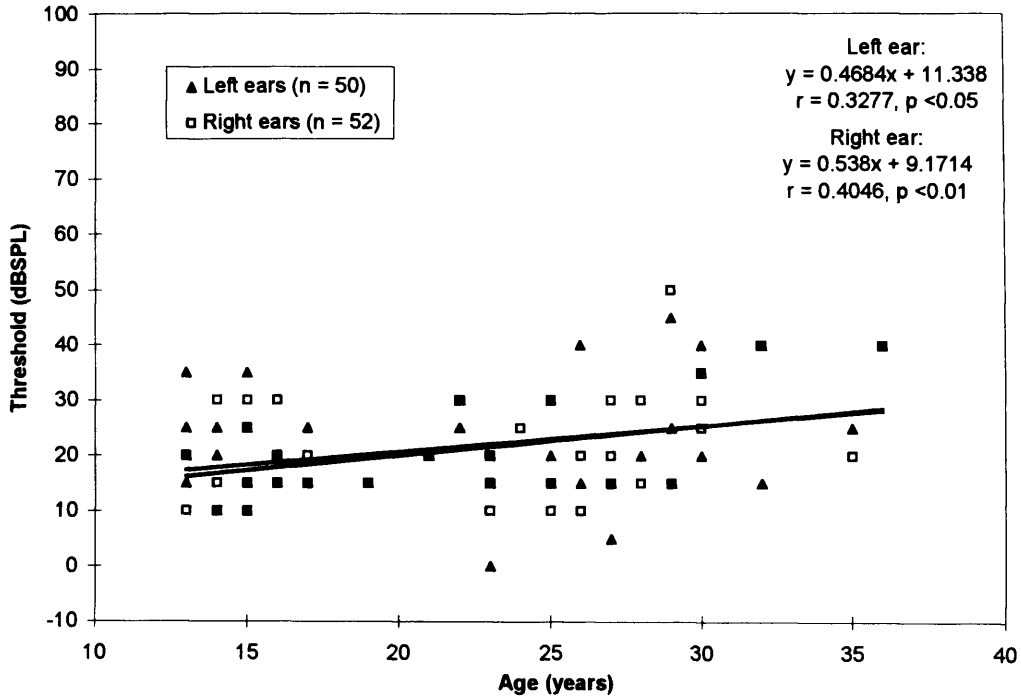


Fig. 3.3.2.1. 10 kHz pure-tone audiometry: Effect of age in controls. Mean threshold+/-SD for left ears = 21.4+/-9.7 dBSPL and right ears = 20.8+/-8.8 dBSPL.

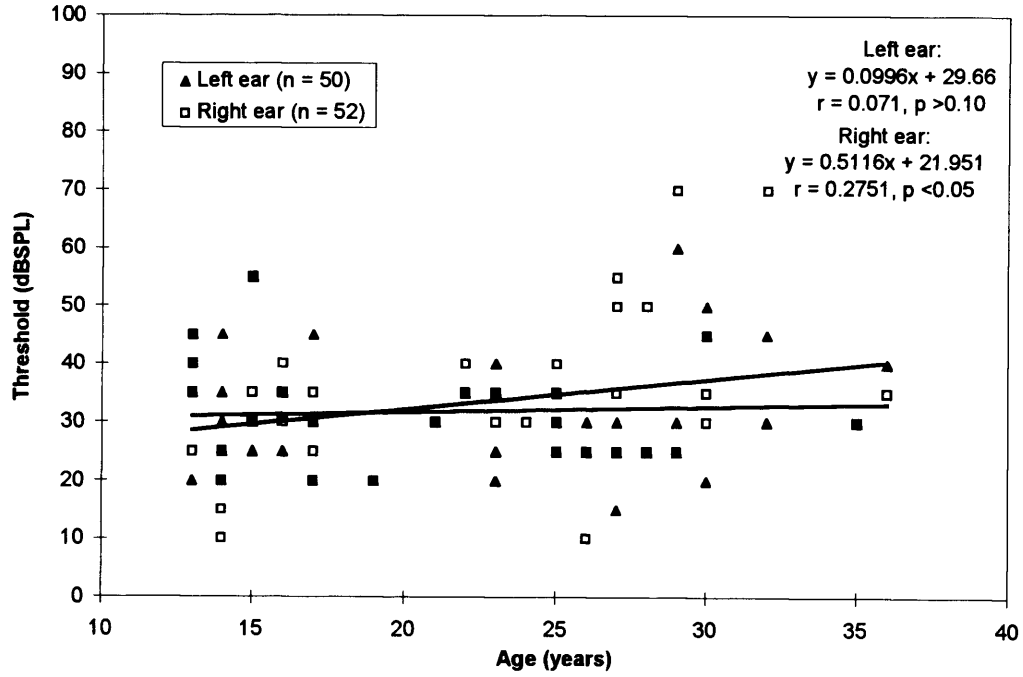


Fig. 3.3.2.2. 12 kHz pure-tone audiometry: Effect of age in controls. Mean threshold+/-SD for left ears = 31.8+/-9.6 dBSPL and right ears = 33.0+/-12.3 dBSPL.

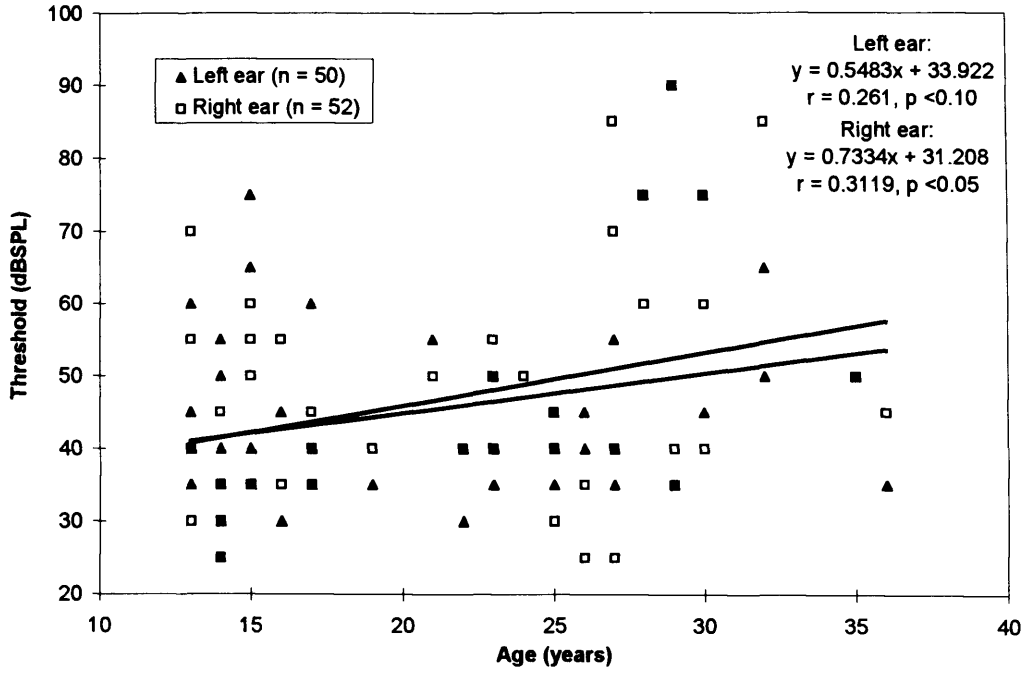


Fig. 3.3.2.3. 14 kHz pure-tone audiometry: Effect of age in controls. Mean threshold+/-SD for left ears = 45.7+/-14.3 dBSPL and right ears = 47.0+/-15.6 dBSPL.

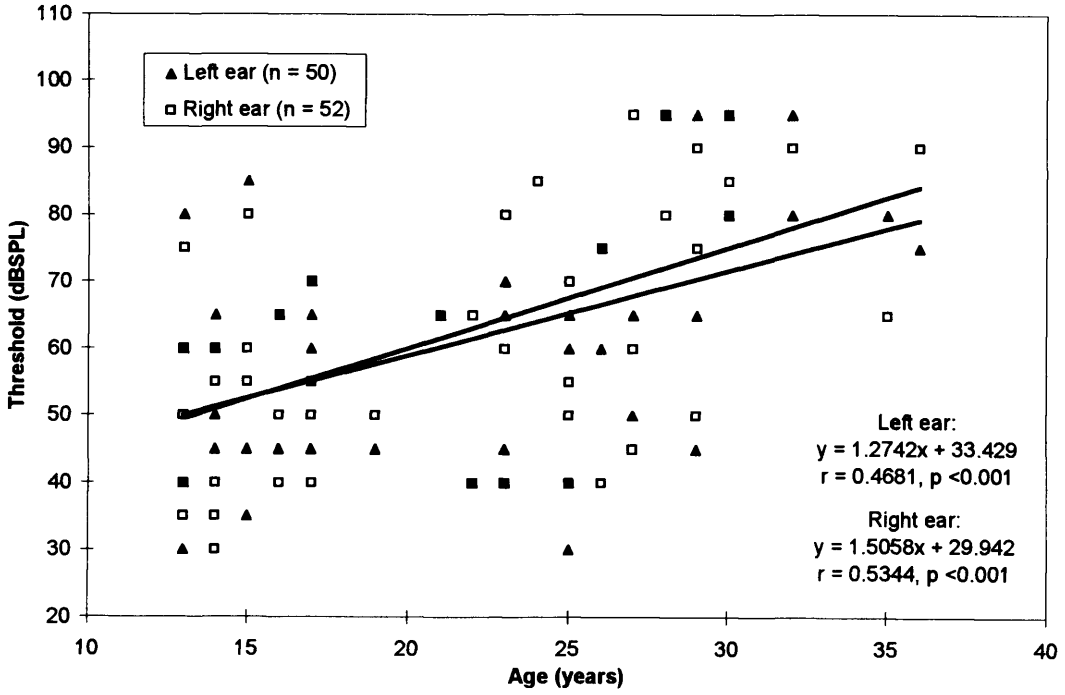


Fig. 3.3.2.4. 16 kHz pure-tone audiometry: Effect of age in controls. Mean threshold+/-SD for left ears = 60.8+/-18.6 dBSPL and right ears = 62.4+/-18.7 dBSPL.

In addition, the median thresholds in dBSPL for the high frequency pure tones - of the control group (comprising 50 left and 52 right ears) - can be taken as 0 dBHL. Hence, RETSPL values (with 5 dB step sizes, and the same for either ear) were: 10 kHz = 20 dBSPL, 12 kHz = 30 dBSPL, 14 kHz = 40 dBSPL, and 16 kHz = 60 dBSPL.

3.3.3. EFFECT OF DOSE

High frequency pure tone thresholds at 10, 12, 14 and 16 kHz were found not to correlate with dose in patients ($p > 0.10$). Dose-response scatter plots and regression analyses are shown, for all four frequencies in Figures 3.3.3.1., 3.3.3.2., 3.3.3.3. and 3.3.3.4. respectively (in all cases Figure A. shows left ears and B. shows right ears). Hence there was no need to partition groups according to dose. Furthermore, no obvious clustering of patients exposed to high peak serum levels was observed. However, patients with sensorineural loss were separated from the distribution of other patient responses, and the dissociation decreased with increasing frequency. The thresholds of patients with sensorineural loss were elevated at all four frequencies to a similar level (95-100 dBSPL); 95-100 dBSPL was within the limits of the test equipment and suggests a saturation in auditory deficit.

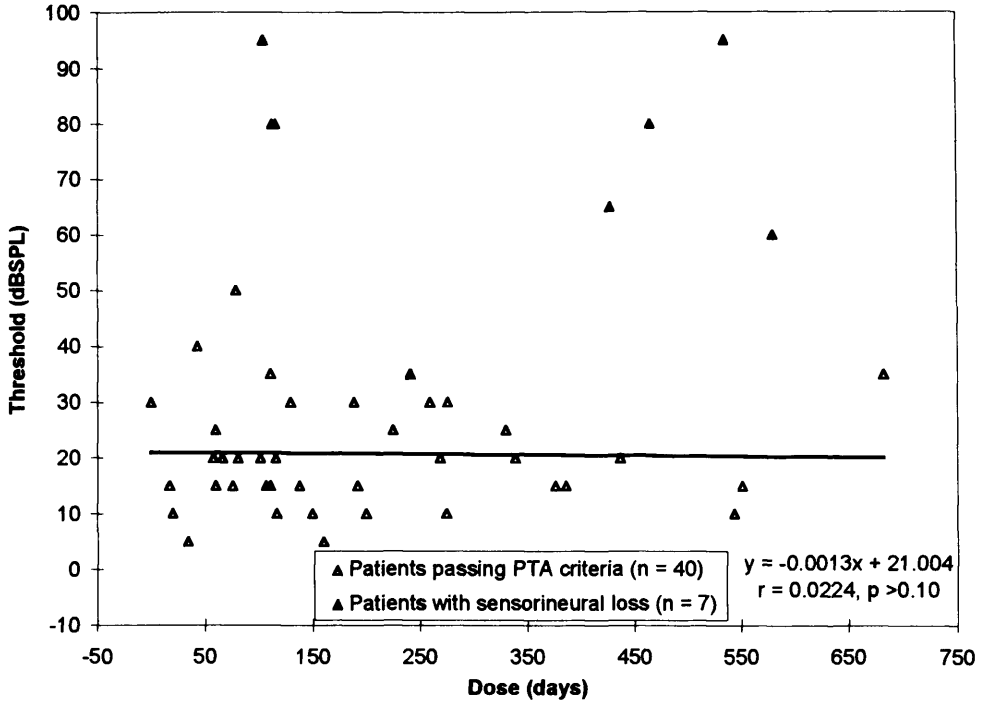


Fig. 3.3.3.1.A. 10 kHz pure-tone audiometry: Dose-response for left ears. Mean threshold+/-SD = 20.8+/-10.0 dBSPL (n = 40).

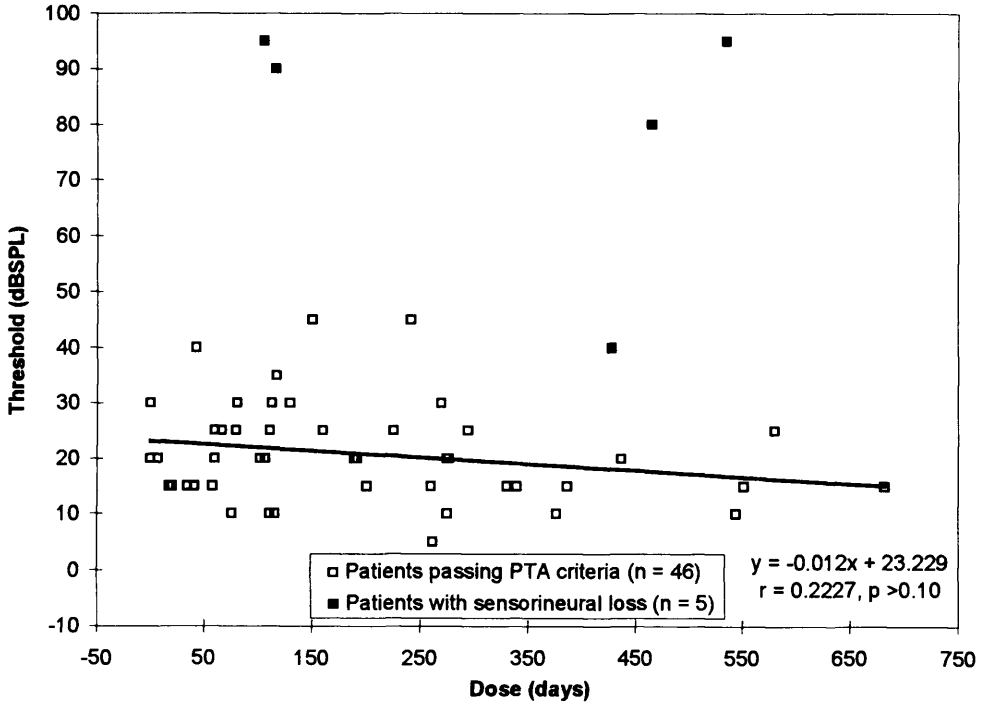


Fig. 3.3.3.1.B. 10 kHz pure-tone audiometry: Dose-response for right ears. Mean threshold+/-SD = 20.9+/-9.0 dBSPL (n = 46).

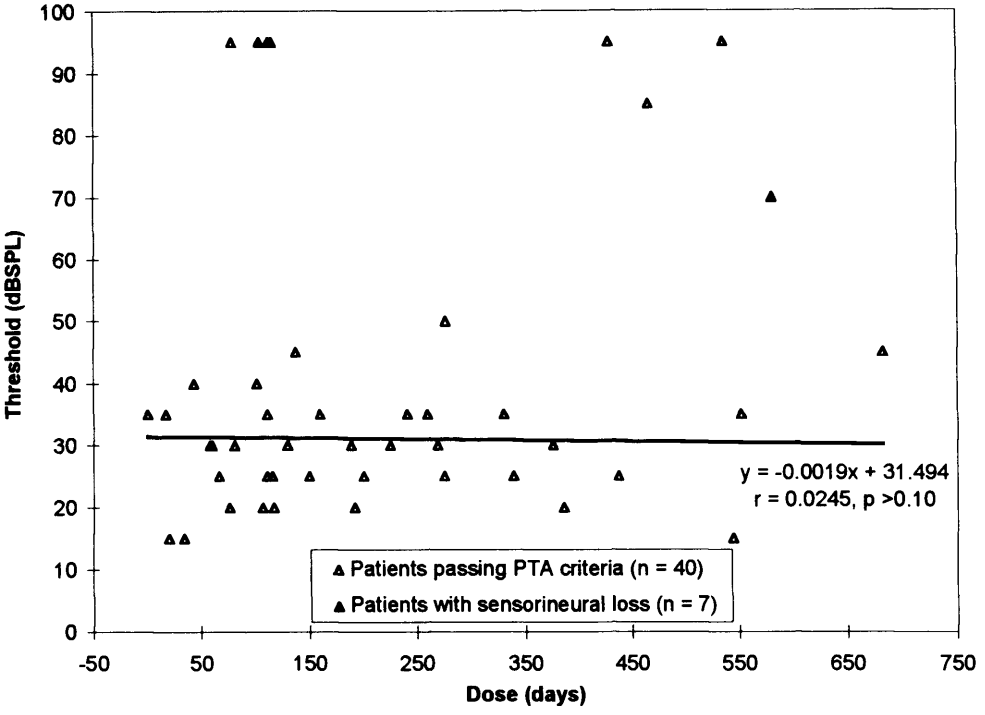


Fig. 3.3.3.2.A. 12 kHz pure-tone audiometry: Dose-response for left ears. Mean threshold+/-SD = 31.1+/-13.2 dBSPL (n = 40).

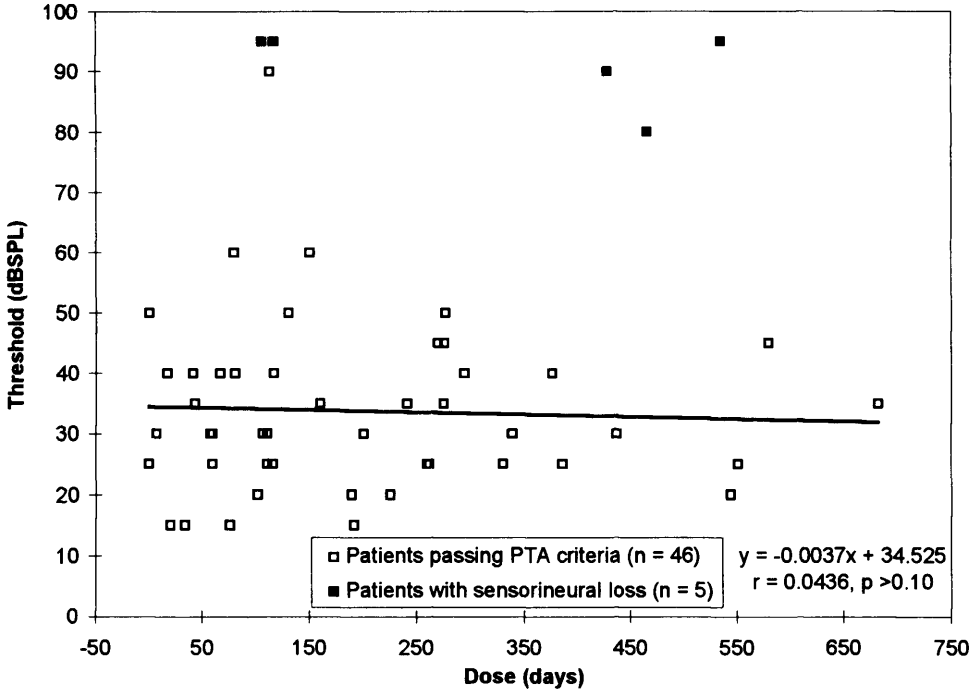


Fig. 3.3.3.2.B. 12 kHz pure-tone audiometry: Dose-response for right ears. Mean threshold+/-SD = 33.8+/-14.1 dBSPL (n = 46).

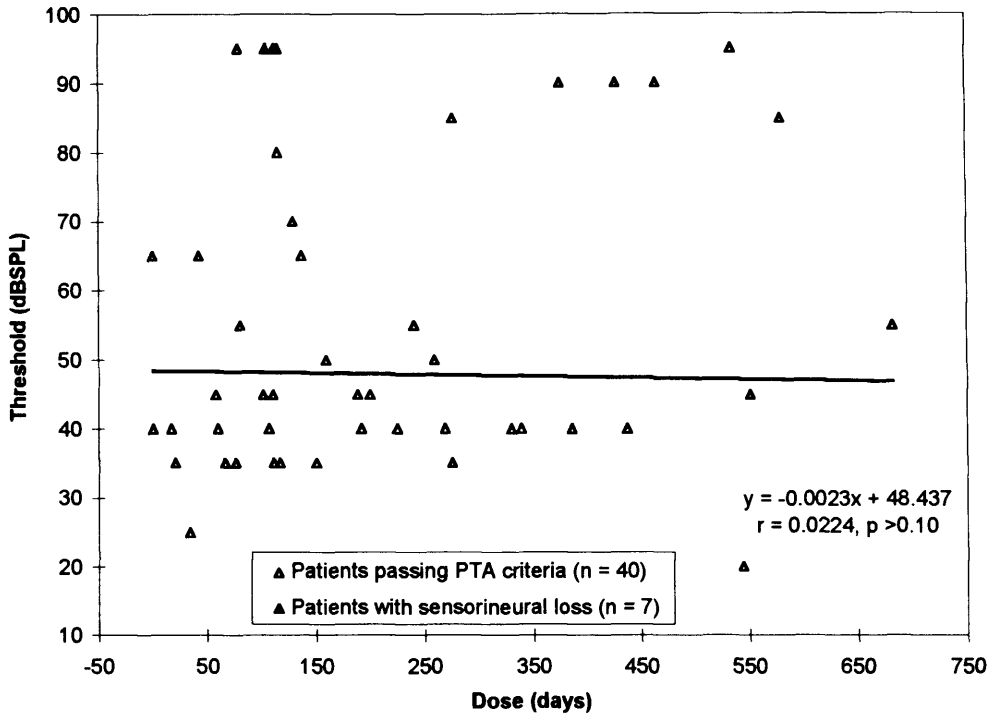


Fig. 3.3.3.3.A. 14 kHz pure-tone audiometry: Dose-response for left ears. Mean threshold+/-SD = 48.0+/-16.9 dBSPL (n = 40).

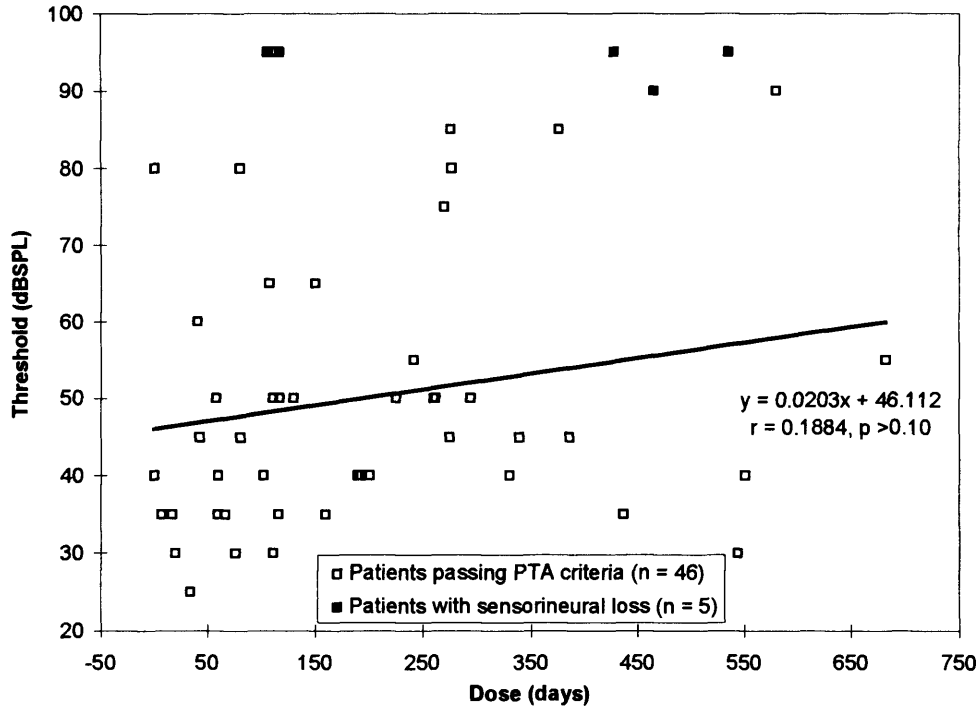


Fig. 3.3.3.3.B. 14 kHz pure-tone threshold: Dose-response for right ears. Mean threshold+/-SD = 50.1+/-18.1 dBSPL (n = 46).

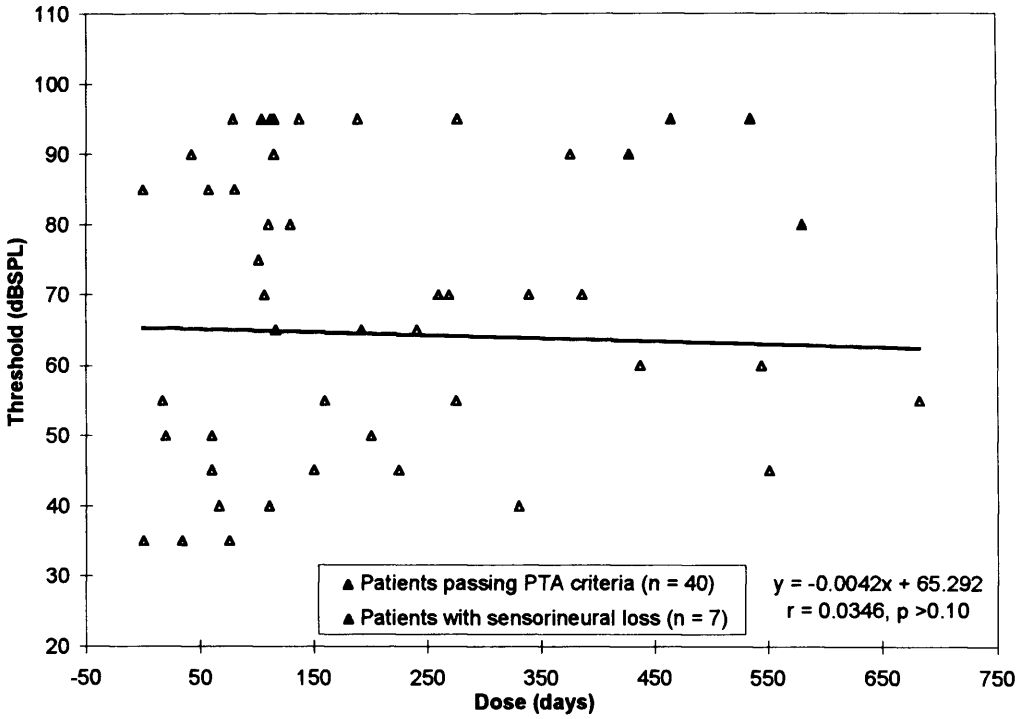


Fig. 3.3.3.4.A. 16 kHz pure-tone audiometry: Dose-response for left ears. Mean threshold+/-SD = 64.5+/-19.4 dBSPL (n = 40).

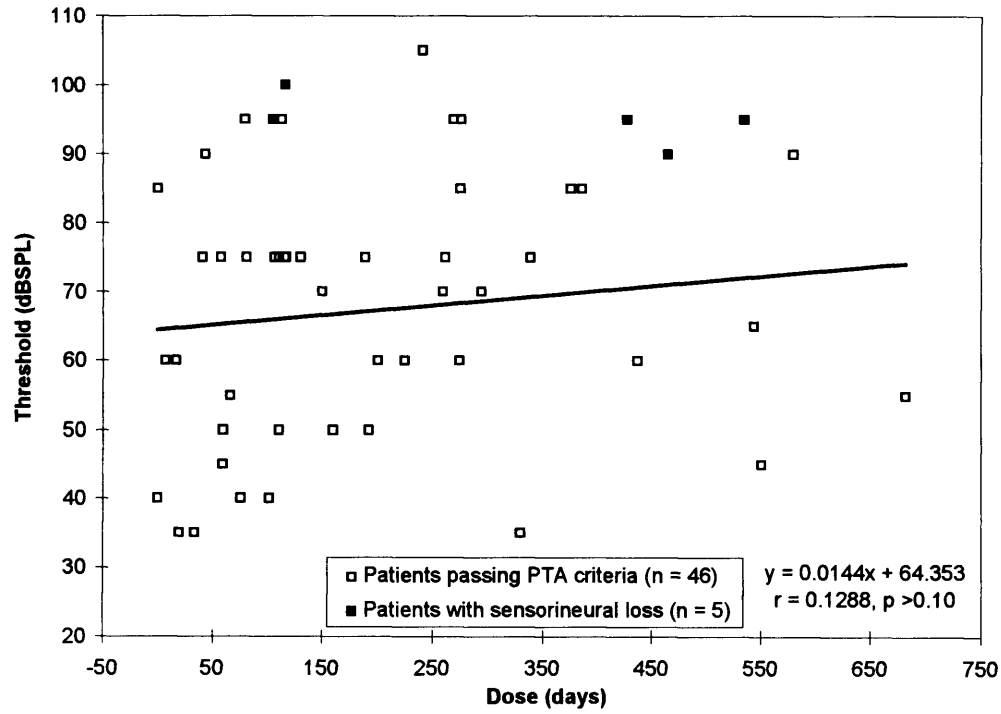


Fig. 3.3.3.4.B. 16 kHz pure-tone audiometry: Dose-response for right ears. Mean threshold+/-SD = 67.2+/-18.6 dBSPL (n = 46).

3.3.4. CONTROLS VERSUS PATIENTS

For high frequency pure tone thresholds (at 10, 12, 14 and 16 kHz), patients and control subjects were both separated into juvenile and adult groups for comparison (see results 3.3.2.). When excluding patients who had received no aminoglycoside exposure and also patients with bilateral sensorineural loss, there were no significant differences in mean threshold ($p > 0.10$) between patients and control subjects in either age group. However when including patients with bilateral sensorineural loss there was a tendency towards significantly higher threshold values in patients, although only for adults and left ears (10 kHz $p = 0.080$, 12 kHz $p = 0.027$, 14 kHz $p = 0.056$, and 16 kHz $p = 0.043$). (See Table 3.3.4..). Furthermore when pooling juvenile and adult groups, including patients with bilateral sensorineural loss did reveal a pattern of inter-supporting differences in variance. At 10 kHz and 12 kHz, patients had a very significantly higher variance (both ears $p > 0.001$ at both frequencies) compared with control subjects. Whereas at 14 kHz patients had a less significantly higher variance (left ear $p = 0.005$, right ear $p = 0.05$), and at 16 kHz there was no significant difference in variance ($p > 0.05$) compared with control subjects.

TABLE. 3.3.4. (overleaf) Comparison of control and patient groups for high frequency PTA (at 10, 12, 14 and 16 kHz): The only significant differences ($p < 0.05$) in mean threshold were for left ears in adults and then only when including patients with bilateral sensorineural loss (see prior text).

Frequency	Group		Left ear threshold mean + /-SD (n) in dBSPL	Right ear threshold mean + /-SD (n) in dBSPL
10 kHz	Controls	Juveniles	18.97 + /-7.1 (23)	17.8 + /-6.2 (23)
		Adults	23.5 + /-11.3 (27)	23.1 + /-10.0 (29)
	Patients without loss	Juveniles	18.3 + /-7.9 (18) Type II T, p = 0.805	20.0 + /-9.6 (19) Type II T, p = 0.380
		Adults	22.0 + /-11.6 (20) Type II T, p = 0.654	20.7 + /-9.1 (23) Type II T, p = 0.364
	Patients + those with loss	Juveniles	18.3 + /-7.9 (18) Type II T, p = 0.805	20.0 + /-9.6 (19) Type II T, p = 0.380
		Adults	34.2 + /-27.5 (25) Type III T, p = 0.080	31.3 + /-26.1 (28) Mann-Whitney, p = 0.668
12 kHz	Controls	Juveniles	31.5 + /-9.5 (23)	29.8 + /-10.6 (23)
		Adults	32.0 + /-9.8 (27)	35.5 + /-13.2 (29)
	Patients without loss	Juveniles	27.2 + /-6.9 (18) Type II T, p = 0.114	29.2 + /-10.7 (19) Type II T, p = 0.863
		Adults	34.3 + /-17.0 (20) Mann-Whitney, p = 0.922	34.4 + /-11.4 (23) Type II T, p = 0.738
	Patients + those with loss	Juveniles	27.2 + /-6.9 (18) Type II T, p = 0.114	29.2 + /-10.7 (19) Type II T, p = 0.863
		Adults	46.0 + /-28.4 (25) Type III T, p = 0.027	44.5 + /-24.5 (28) Type III T, p = 0.095
14 kHz	Controls	Juveniles	42.6 + /-12.7 (23)	42.8 + /-11.3 (23)
		Adults	48.3 + /-15.3 (27)	50.3 + /-17.8 (29)
	Patients without loss	Juveniles	43.9 + /-13.1 (18) Type II T, p = 0.754	43.7 + /-15.4 (19) Type II T, p = 0.836
		Adults	51.3 + /-20.0 (20) Type II T, p = 0.571	50.9 + /-15.2 (23) Type II T, p = 0.911
	Patients + those with loss	Juveniles	43.9 + /-13.1 (18) Type II T, p = 0.754	43.7 + /-15.4 (19) Type II T, p = 0.836
		Adults	59.6 + /-24.5 (25) Type III T, p = 0.056	58.6 + /-21.7 (28) Type II T, p = 0.123
16 kHz	Controls	Juveniles	55.0 + /-15.5 (23)	53.5 + /-14.2 (23)
		Adults	65.7 + /-19.8 (27)	69.5 + /-19.0 (29)
	Patients without loss	Juveniles	56.4 + /-17.6 (18) Type II T, p = 0.790	60.5 + /-20.9 (19) Type II T, p = 0.203
		Adults	72.3 + /-17.3 (20) Type II T, p = 0.246	70.9 + /-13.9 (23) Type II T, p = 0.771
	Patients + those with loss	Juveniles	56.4 + /-17.6 (18) Type II T, p = 0.790	60.5 + /-20.9 (19) Type II T, p = 0.203
		Adults	76.6 + /-17.8 (25) Type II T, p = 0.043	75.2 + /-15.7 (28) Type II T, p = 0.224

TABLE. 3.3.4. (legend on previous page).

Etymotic earphone insertion depths for control subjects and patients were not significantly different ($p > 0.10$). Where, mean+/-SD for left ears = 4.6+/-2.6 mm (n = 46 control ears) versus 4.8+/-3.5 mm (n = 45 patient ears, Mann-Whitney test, $p = 0.606$); and mean+/-SD for right ears = 4.5+/-2.9 mm (n = 49 control ears) versus 4.2+/-3.4 mm (n = 49 patient ears, Mann-Whitney test, $p = 0.365$).

Retrospective power analysis for threshold (dBSPL) reveals that with a mean standard deviation of 12.7 dB (pooling control left and right ears over all four frequencies), for 80% power and $p = 0.05$: to detect a difference of 10 dB between means, each group needed to include 26 measures; to detect a difference of 11 dB between means, each group needed to include 21 measures; and to detect a difference of 12 dB between means, each group needed to include 18 measures. The actual number of patient measures (18-25 left, and 19-28 right ears; depending on hypothesis) and control measures (23-27 left and 23-29 right ears; similarly depending on hypothesis) can therefore be expected to have detected a significant difference of 11 dB (at $p = 0.05$) in 80 % of cases.

Hence, for all four frequencies, sample size was sufficient to detect a difference between patient and control groups of 11 dB. Significant differences ($p < 0.05$) in mean threshold were only apparent for adult left ears, and then only at 12 and 16 kHz and when including patients with bilateral sensorineural loss. As neither both ears nor adjacent frequencies exhibit significant differences, these findings were not considered to constitute a consistent alteration in mean threshold. In addition significant differences in variance were found, these were greatest at the lower frequencies of 10 kHz and 12 kHz ($p < 0.001$), and decreased with increasing frequency (14 kHz $p = 0.05$-0.005), with there being no significant difference in variance at 16 kHz ($p > 0.05$).

3.4. FREQUENCY RESOLUTION

All frequency resolution data was first interpreted by calculating the difference between thresholds for the two extremes of no notch and the widest notch masking conditions. A second approach, by fitting the data to a simple computer model (the ro-ex(p) expression: for derivation of rounded exponential (p), K, and, ERB), was not performed as a working copy of the computer program was unavailable.*

With respect to frequency resolution measurements, all absolute threshold values were presented in dBSPL (recorded +/-1.0 dB).** The level of masking varied slightly between earpiece and frequency (see Table 3.4.), but remained within an overall level of 68-72 dBSPL and a spectrum level of 50-56 dBSPL/Hz (as stipulated in the methods 2.4.1.).

Frequency	Type of masking measurement	Earpiece masking level (in dBSPL)		
		Left	Right	Combined mean
2 kHz	Overall	70-72	71-72	71.25
	Spectrum	55-56	54-55	55
4 kHz	Overall	69-71	71-72	70.75
	Spectrum	50-52	52	51.5
8 kHz	Overall	70	70	70
	Spectrum	54	54	54

TABLE. 3.4. Values of masking level for frequency resolution: Ranges represent slight differences in calibration measurements between the start and end of the study.

* Where significant differences dictated a more detailed investigation a more thorough analysis is expected in the near future.

** For 2, 4 and 8 kHz pure tones, 0 dBHL is equivalent to 8.4, 9.3, and 11.5 dBSPL respectively (BS 2497:1992).

3.4.1. TEST RE-TEST VARIABILITY

For frequency resolution (difference between masking extremes at 2, 4 and 8 kHz), repeated measurements in one representative control individual were found to be approximately equally stable, irrespective of the time of testing (see Table 3.4.1.).

Frequency	Test interval	Left ear difference mean +/-SD (dB)	Right ear difference mean +/-SD (dB)
2 kHz	AM-PM	Not tested	19.9 +/-3.0
	Month-Month	21.5 +/-1.7	19.4 +/-3.5
4 kHz	AM-PM	Not tested	34.4 +/-2.1
	Month-Month	27.5 +/-2.2	28.1 +/-3.2
8 kHz	AM-PM	33.9 +/-1.8	30.6 +/-1.2
	Month-Month	32.6 +/-1.9	32.6 +/-1.5

TABLE. 3.4.1. Test-retest variability of frequency resolution (difference between masking extremes at 2, 4 and 8 kHz): AM-PM over one week (n = 5 days AM and PM) and month-month over one year (n = 12 months).

3.4.2. EFFECT OF AGE

Frequency resolution measurements (difference (dB) between masking extremes) at 4 and 8 kHz were found to correlate with age in control subjects ($p > 0.05$) for both ears. However, at 2 kHz there was no correlation ($p > 0.10$) in either ear. Hence when comparing patients and control subjects, it was considered necessary only to partition groups according to age for 4 and 8 kHz. Scatter plots and regression analyses are shown, for all three frequencies in Figures 3.4.2.1., 3.4.2.2. and 3.4.2.3. respectively.

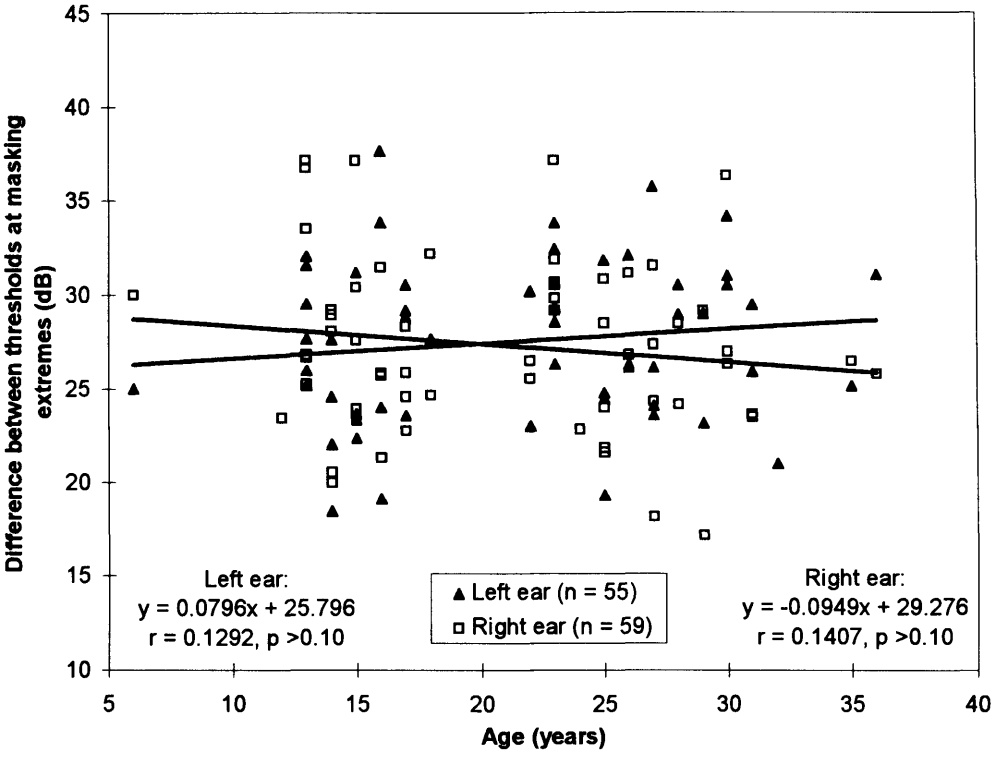


Fig. 3.4.2.1. 2 kHz frequency resolution: Effect of age in controls. Mean difference+/-SD for left ears = 27.5+/-4.3 dB and right ears = 27.3+/-4.6 dB.

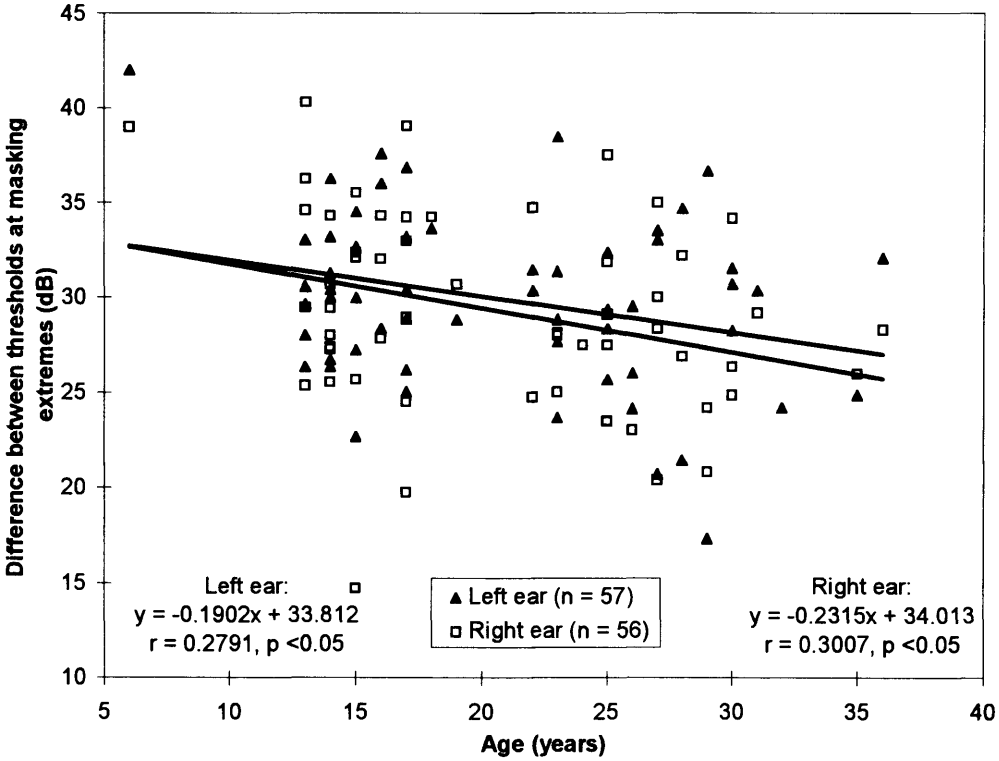


Fig. 3.4.2.2. 4 kHz frequency resolution: Effect of age in controls. Mean difference+/-SD for left ears = 29.9+/-4.7 dB and right ears = 29.2+/-5.2 dB.

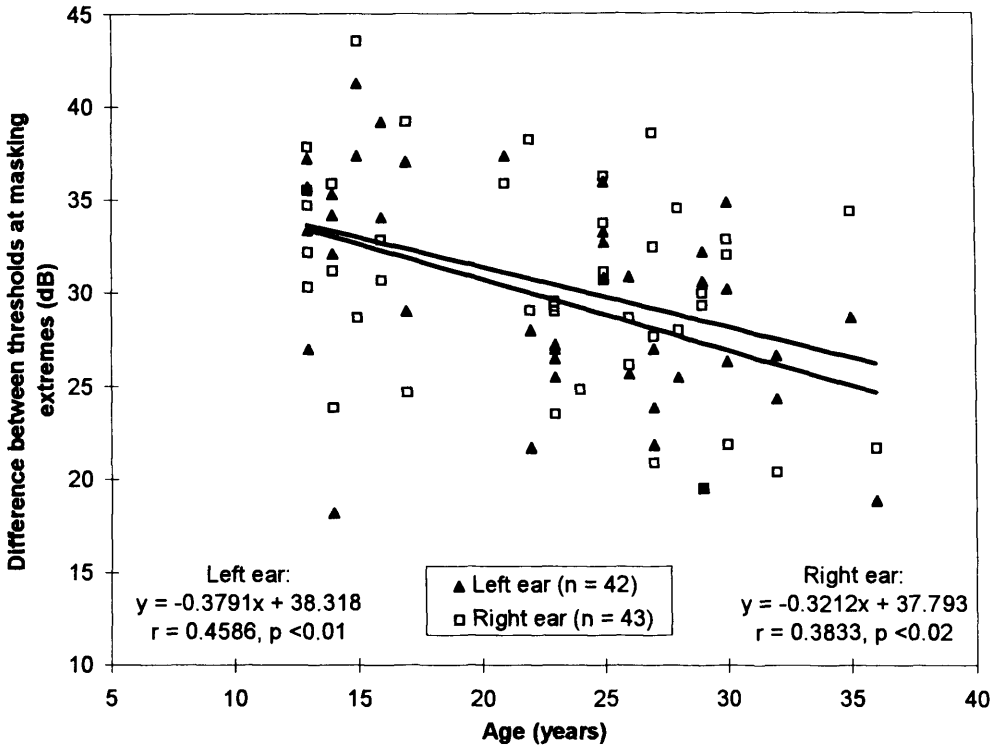


Fig. 3.4.2.3. 8 kHz frequency resolution: Effect of age in controls. Mean difference+/-SD for left ears = 29.8+/-5.7 dB and right ears = 30.5+/-5.6 dB.

3.4.3. EFFECT OF DOSE

Frequency resolution measurements (difference (dB) between masking extremes) at 2, 4 and 8 kHz were found not to correlate with dose in patients ($p > 0.10$). Dose-response scatter plots and regression analyses are shown, for all three frequencies in Figures 3.4.3.1., 3.4.3.2. and 3.4.3.3. respectively (in all cases Figure A. shows left ears and B. shows right ears). Hence there was no need to partition groups according to dose. Furthermore, there was no obvious clustering of patients either exposed to high peak serum levels, or with sensorineural loss. However, the worst frequency resolution measurements were associated with sensorineural loss.

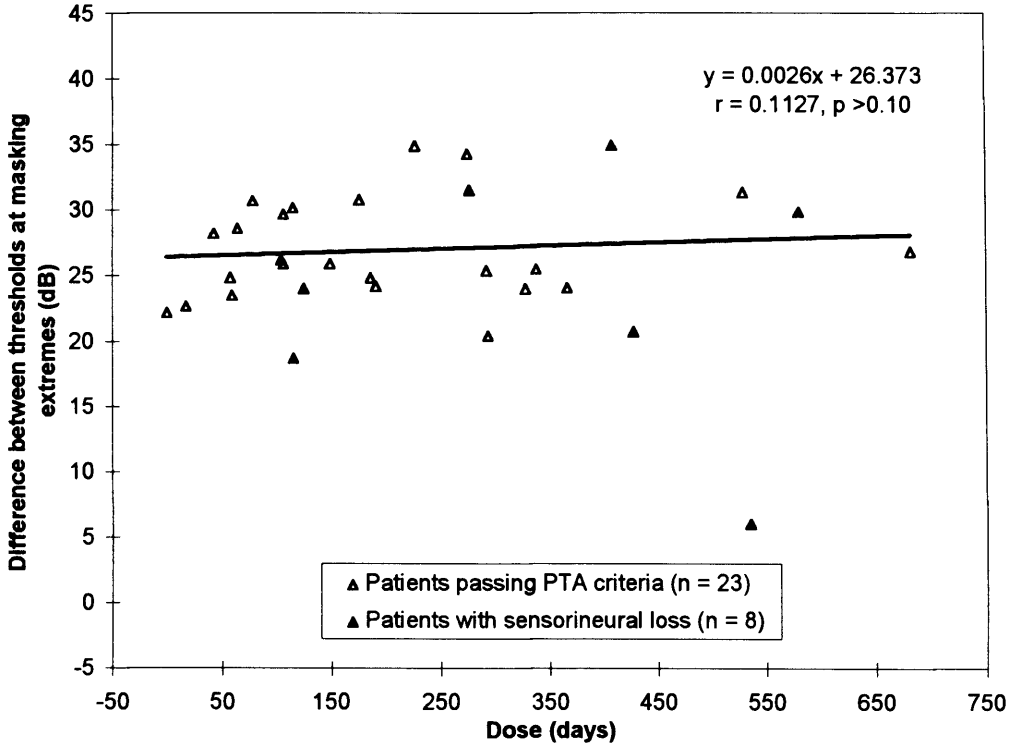


Fig. 3.4.3.1.A. 2 kHz frequency resolution: Dose-response for left ears. Mean difference+/-SD = 26.9+/-3.8 dB (n = 23).

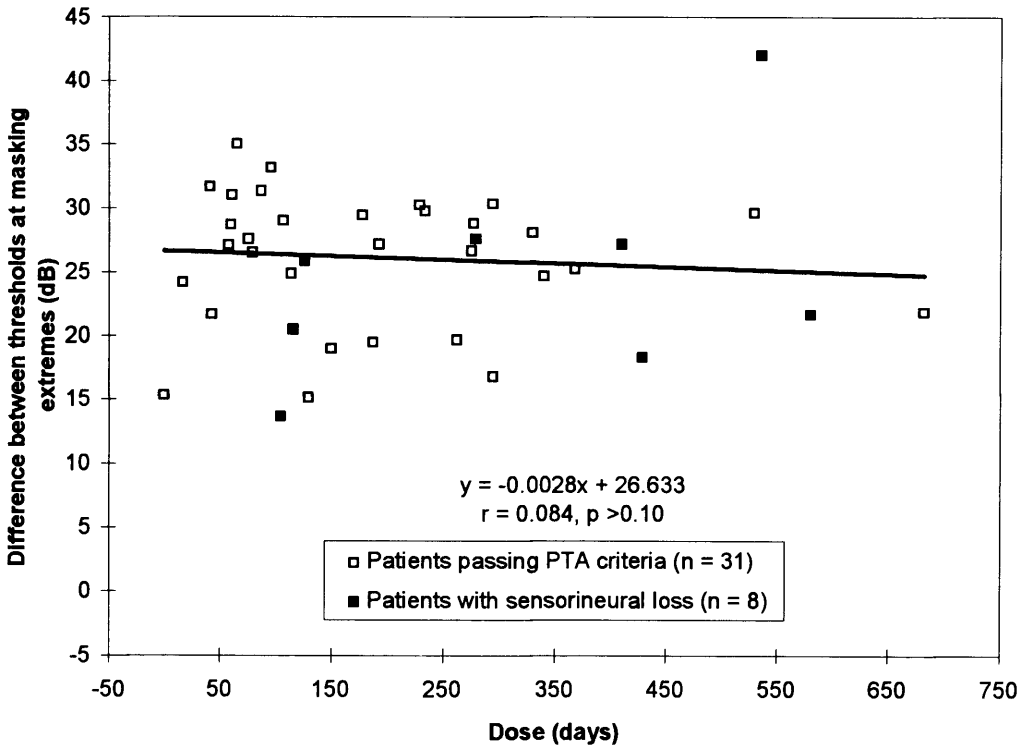


Fig. 3.4.3.1.B. 2 kHz frequency resolution: Dose-response for right ears. Mean difference+/-SD = 26.1+/-5.2 dB (n = 31).

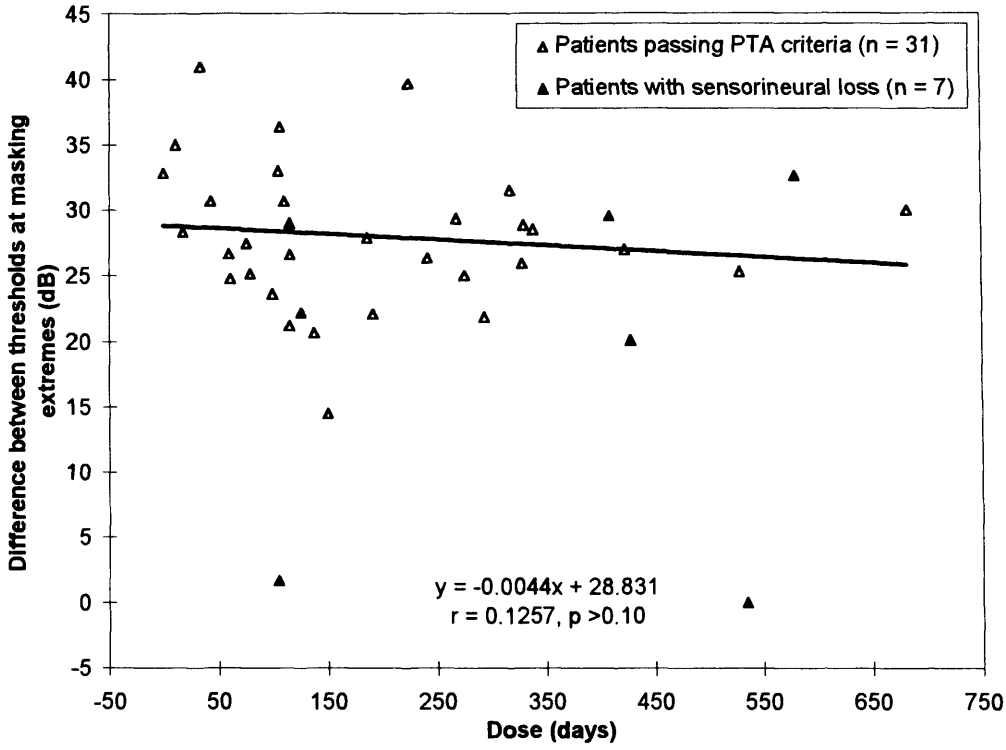


Fig. 3.4.3.2.A. 4 kHz frequency resolution: Dose-response for left ears. Mean difference+/-SD = 28.0+/-5.6 dB (n = 31).

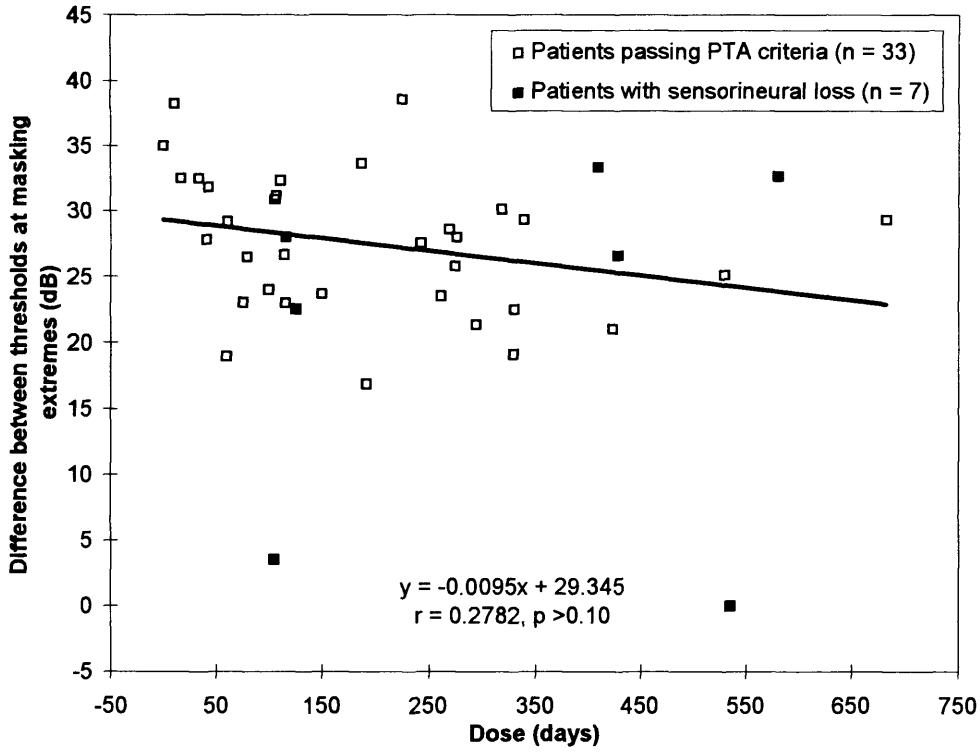


Fig. 3.4.3.2.B. 4 kHz frequency resolution: Dose-response for right ears. Mean difference+/-SD = 27.5+/-5.4 dB (n = 33).

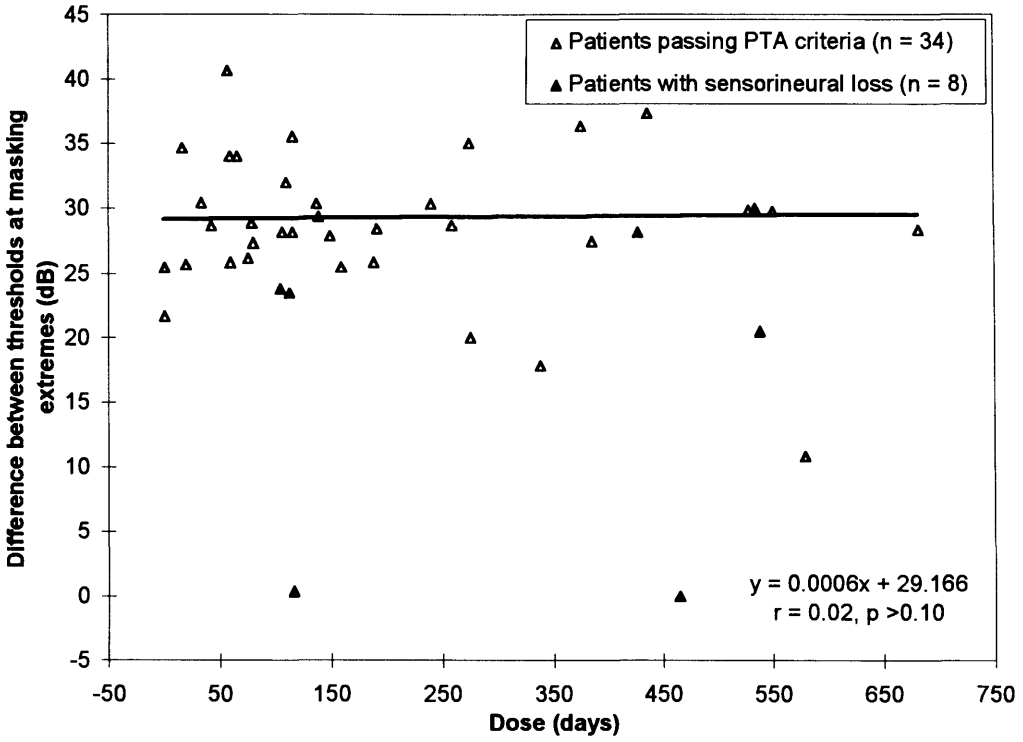


Fig. 3.4.3.3.A. 8 kHz frequency resolution: Dose-response for left ears. Mean difference+/-SD = 29.3+/-4.8 dB (n = 34).

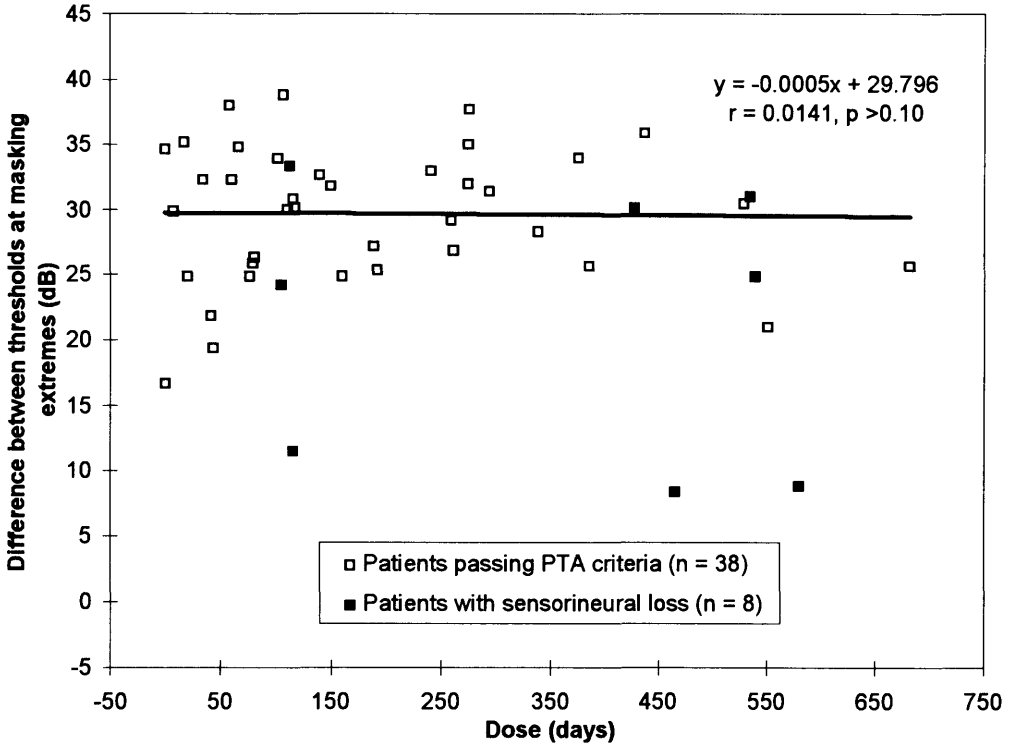


Fig. 3.4.3.3.B. 8 kHz frequency resolution: Dose-response for right ears. Mean difference+/-SD = 29.7+/-5.3 dB (n = 38).

3.4.4. CONTROLS VERSUS PATIENTS

For frequency resolution (difference between masking extremes) at 4 and 8 kHz, patients and control subjects were both separated into juvenile and adult groups for comparison (see results 3.4.2.). When excluding patients who had received no aminoglycoside exposure and either excluding or including patients with bilateral sensorineural loss, there was only one significant difference ($p > 0.05$) between patients and control subjects. At 4 kHz when including patients with bilateral sensorineural loss, patients had a significantly smaller mean difference than control subjects, although only for adult left ears ($p = 0.025$). (See Table 3.4.4..)

Retrospective power analysis for difference (dB) between masking extremes, reveals that with a mean standard deviation of 4.9 dB (pooling control left and right ears over all three frequencies), for 80% power and $p = 0.05$: to detect a change of 4 dB between means, each group needed to include 24 measures; to detect a change of 5 dB between means, each group needed to include 16 measures; and to detect a change of 6 dB between means, each group needed to include 12 measures. The actual number of patient measures (12-28 left, and 14-36 right ears; depending on hypothesis) and control measures (15-55 left and 14-59 right ears; similarly depending on hypothesis) can therefore be expected to have detected a significant difference of 5 dB (at $p = 0.05$) in 80 % of cases.

Hence, for all three frequencies, sample size was sufficient to detect a change between patient and control groups of 5 dB in difference between masking extremes. Only at 4 kHz and when including patients with bilateral sensorineural loss, was frequency resolution significantly poorer in patients when compared with control subjects ($p = 0.025$) and then only for left ears in adults.

Frequency	Group		Left ear difference mean+/-SD (n) in dB	Right ear difference mean+/-SD (n) in dB
2 kHz	Controls		27.5+/-4.3 (55)	27.3+/-4.6 (59)
	Patients, excluding those with loss		27.1+/-3.8 (22) Type II T, p=0.701	26.5+/-4.9 (30) Type II T, p=0.435
	Patients, including those with loss		26.2+/-5.8 (28) Mann-Whitney, p=0.441	26.2+/-5.9 (36) Type II T, p=0.315
4 kHz	Controls	Juveniles	30.9+/-4.3 (29)	30.6+/-5.7 (29)
		Adults	28.8+/-4.9 (28)	27.8+/-4.3 (27)
	Patients without loss	Juveniles	29.7+/-5.6 (15) Type II T, p=0.429	28.4+/-5.6 (15) Type II T, p=0.242
		Adults	26.0+/-5.1 (15) Type II T, p=0.084	26.3+/-4.9 (17) Type II T, p=0.282
	Patients + those with loss	Juveniles	29.7+/-5.6 (15) Type II T, p=0.429	28.4+/-5.6 (15) Type II T, p=0.242
		Adults	23.5+/-9.1 (20) Type III T, p=0.025	24.5+/-8.7 (22) Mann-Whitney, p=0.330
8 kHz	Controls	Juveniles	33.2+/-5.9 (15)	32.9+/-5.4 (14)
		Adults	27.9+/-4.7 (27)	29.3+/-5.3 (29)
	Patients without loss	Juveniles	31.6+/-4.4 (12) Type II T, p=0.439	31.0+/-4.6 (14) Type II T, p=0.316
		Adults	28.5+/-4.6 (20) Type II T, p=0.676	29.3+/-5.0 (22) Type II T, p=0.983
	Patients + those with loss	Juveniles	31.6+/-4.4 (12) Type II T, p=0.439	31.0+/-4.6 (14) Type II T, p=0.316
		Adults	26.1+/-8.9 (25) Mann-Whitney, p=0.971	27.7+/-6.9 (27) Type II T, p=0.349

TABLE. 3.4.4. Comparison of control and patient groups for frequency resolution (difference between masking extremes at 2, 4 and 8 kHz): The only significant difference ($p > 0.05$) was at 4 kHz, in adult left ears and when including patients with bilateral sensorineural loss (see prior text).

3.5. OTO-ACOUSTIC EMISSIONS - DP-GRAMS

3.5.1. TEST RE-TEST VARIABILITY

For DPOAE test-retest measures the individual tested had no SOAEs or CSSOAEs (fluctuations in which could have confounded DPOAE variability; Wier et al., 1988). As an indicator of DP-gram variability, mean standard deviations were calculated (standard deviations calculated from repeated measurements for each frequency point on the DP-gram were pooled*). For DP-gram $2f_1$-f_2 amplitudes, repeated measurements in one representative individual were found to be stable irrespective of the time of testing between: AM-PM over one week (n = 5 days AM and PM, for 26 frequency points, mean SD+/-SD for right ear = 1.35+/-0.55 dB); and week-week over one year (for 26 frequency points, mean SD+/-SD: left ear = 1.26+/-0.47 dB, n = 43 weeks; right ear = 1.39+/-0.60 dB, n = 44 weeks).

3.5.2. EFFECT OF AGE

DPOAE input-output functions provide justification against separating DP-grams according to age (see results 3.6.2.).

3.5.3. EFFECT OF DOSE

DPOAE input-output functions also provide justification against separating DP-grams according to dose (see results 3.6.3.).

---

* DP-grams might be expected to show a slightly lower degree of variability at 1 kHz, due to occasional overlap of the DP-gram cycle (for 1001 Hz: left ear weekly mean SD = 1.22 dB, right ear weekly mean SD = 1.07 dB).

3.5.4. CONTROLS VERSUS PATIENTS

Figures 3.5.4.A. and B. show the DP-grams for CF patients (48 left ears and 51 right ears; excluding patients with no aminoglycoside exposure) as mean $2f_1$-f_2, together with the 2 SD limits for control subjects (80 left ears and 79 right ears), mean 2 SD upper limit of the background noise (for control subjects), and the mean level of system distortion (from 4 complete cycles in an artificial 1 cm^3 cavity, measured on separate days). The CF patients with bilateral sensorineural hearing loss (6 left and 6 right ears) are shown as separate mean DP-grams. For patients with bilateral sensorineural loss the $2f_1$-f_2 intensities fall below the lower 2 SD limit of the control values between 3 and 4 kHz.

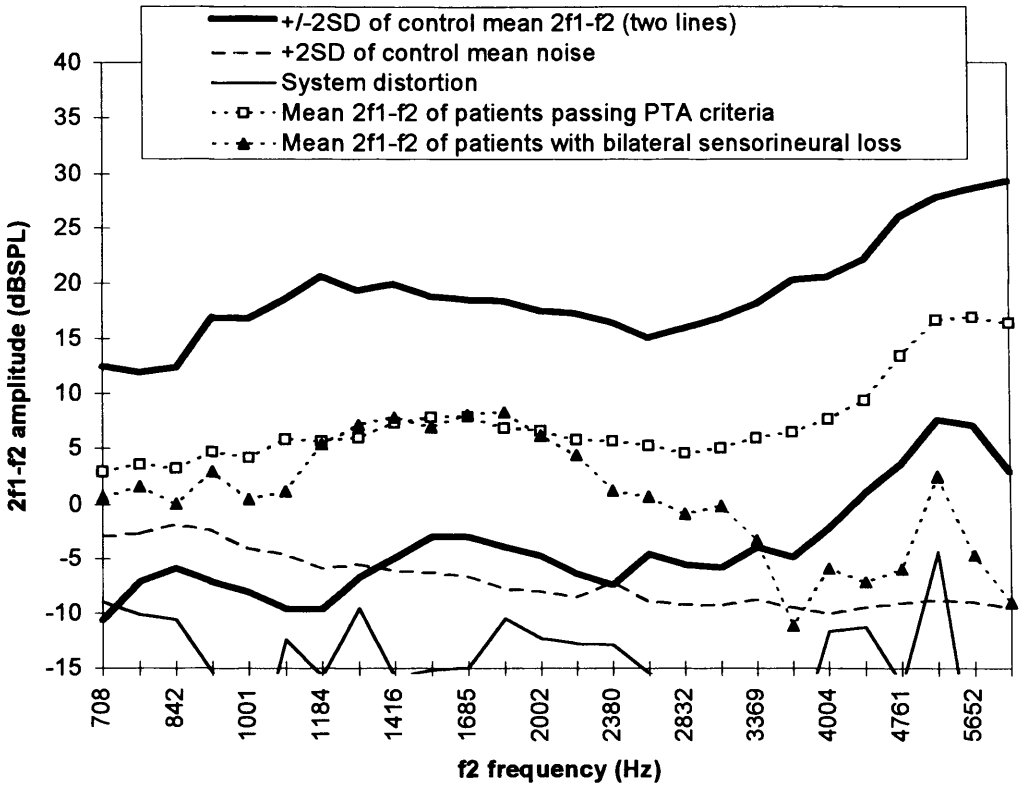


Fig. 3.5.4.A. DP-grams for left ears

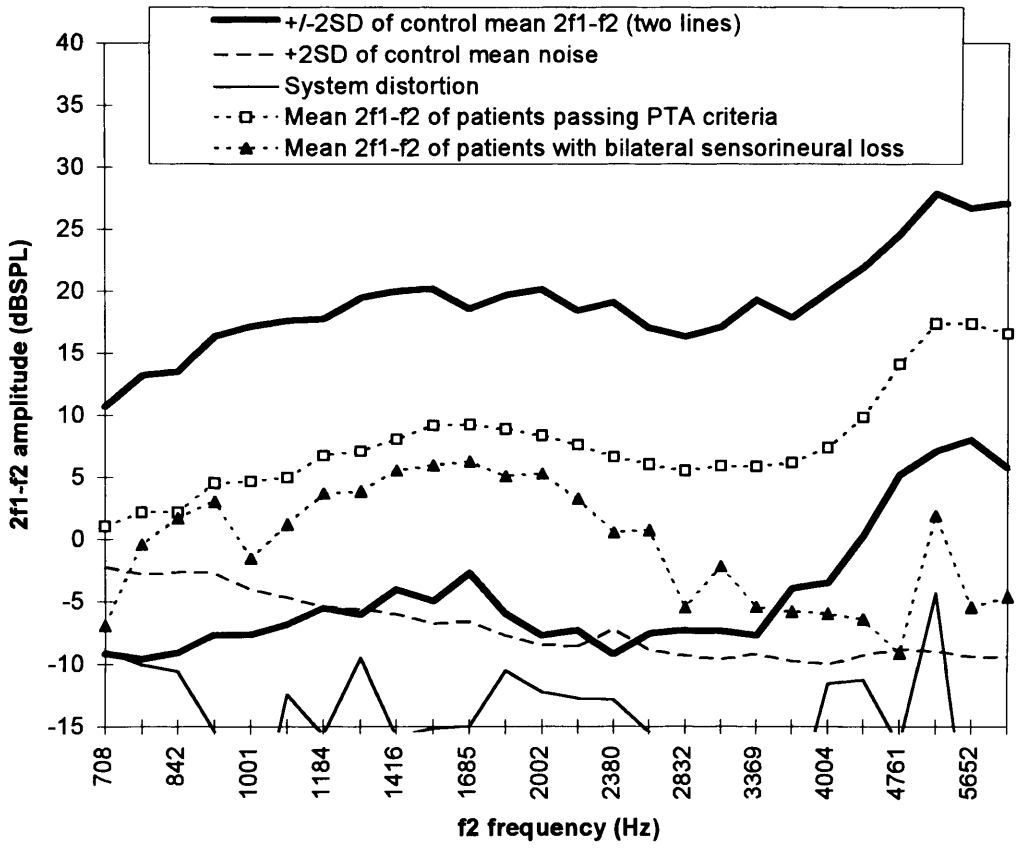


Fig. 3.5.4.B. DP-grams for right ears

3.6. OTO-ACOUSTIC EMISSIONS - DPOAE INPUT-OUTPUTS

DPOAE input-output functions can follow different shapes in different individuals and at different frequencies. All of the input-output functions observed in the study have fallen into one of the following categories (Figure 3.6. A-H. overleaf; also see Figure 1.8.10.B.):

- Linear = single-segment straight line trajectory.
- Saturating = 3 or more consecutive points deviating from a linear interpolation (i.e. exponential rise to a plateau with increasing stimulus level).
- Flat-steep = 2 distinct linear components (includes hyperbolic shapes).
- Diphasic = 2 distinct components joined by a depressed response.
- Non-monotonic = 2 distinct non-linear components joined by an elevated response (includes parabolic shapes).
- None = less than 2 consecutive points 2 SD above noise (sometimes flat).
- Passive = points only seen with a stimulus above 65 dBSPL.
- Noisy = 2 SD of noise was above -5 dBSPL for at least one point (and that input-output function measure was then excluded).

Input-output function shapes, iso-criteria* and slopes were designated according to the following criteria (over the stimulus range of 35 to 70 dBSPL): one point was insufficient to influence designation; any points below -10 dBSPL were insufficient to influence designation; input-output functions with an iso-criterion below the minimum stimulus level of 35 dBSPL were extrapolated down to a theoretical limit** of 20

---

* Iso-criteria were measured as the stimulus level (in dBSPL) that corresponded to the point at which the line of the input-output function crossed the DPOAE response level of -10 dBSPL.

** The lowest possible iso-criterion designation was decided by applying the extrapolation criteria equally and without limitation to all input-output functions. A theoretical stimulus level of 20 dBSPL was considered to correspond to the minimum limit that could still maintain a continuous and normal distribution (see methods 2.6.3.).

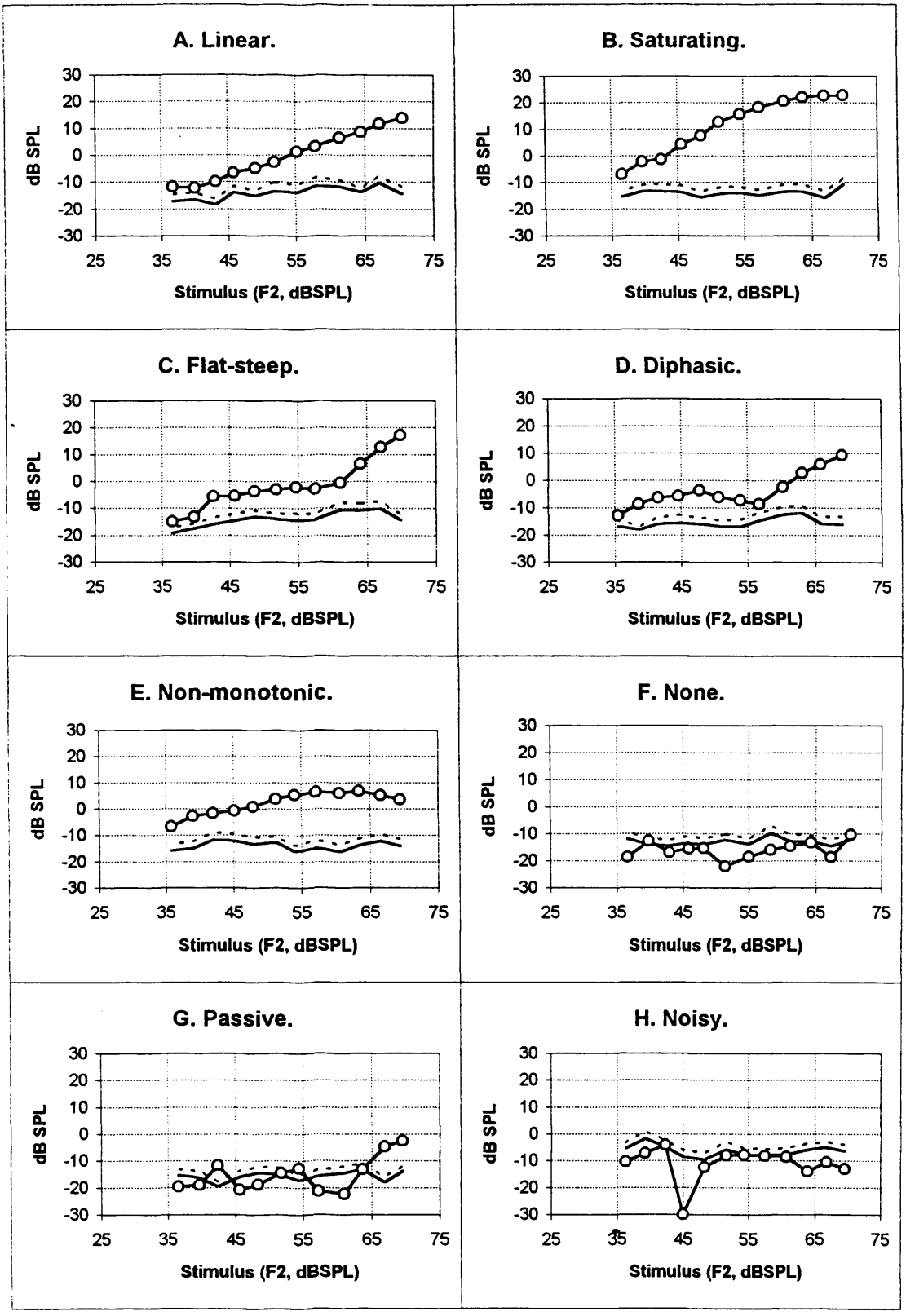


Fig. 3.6. An example DPOAE input-output function recording for each category of shape of input-output function: **A** = Linear; **B** = Saturating; **C** = Flat-steep; **D** = Diphasic; **E** = Non-monotonic; **F** = None; **G** = Passive; and, **H** = Noisy. Circles represent mean $2f_1$-f_2 amplitude, with continuous and dotted lines representing +1 SD and +2 SD respectively.

dBSPL; and, when presented with a choice, the lowest iso-criterion or 'worst' shape (indicative of the fewest generator sites) was always selected for consistency.

An iso-criterion at -10 dBSPL was measured for all included input-output functions. For linear and flat-steep input-output functions the overall slope (Y/X) was also measured. For further comparison, shapes were grouped into categories indicative of the number of interacting factors that was thought might be contributing to the pattern observed. Linear, saturating, passive and none were all assumed to reflect one or less generator site (single generator site). Whereas, flat-steep, non-monotonic and diphasic were assumed to reflect more than one generator site (multiple generator sites).

3.6.1. TEST RE-TEST VARIABILITY

For input-output function -10 dBSPL iso-criteria and slopes (at 2, 4, 6 and 8 kHz), repeated measurements in one representative control individual were found to be approximately equally stable, irrespective of the time of testing (see Table 3.6.1.).

Input-output function measurement	Frequency (kHz)	Left ear Mean+/-SD (n)	Right ear Mean+/-SD (n)
-10 dBSPL iso-criterion (in dBSPL)	2	55.60+/-3.31 (42)	43.80+/-8.80 (44)
	4	46.91+/-1.72 (42)	51.61+/-1.79 (44)
	6	38.34+/-4.82 (44)	38.52+/-3.66 (44)
	8	60.14+/-1.73 (43)	57.57+/-6.28 (37)
Slope (Y/X as dB_{in} / dB_{out})	2	0.58+/-0.22 (34)	0.62+/-0.24 (29)
	4	1.09+/-0.08 (44)	1.26+/-0.11 (44)
	6	1.00+/-0.06 (44)	1.08+/-0.07 (33)
	8	2.07+/-0.34 (43)	2.87+/-0.52 (29)

TABLE. 3.6.1. Week-week test-retest variability (over one year) of input-output function -10 dBSPL iso-criteria and slopes (at 2, 4, 6 and 8 kHz).

For all four frequencies, repeated measurements in one representative individual did not reveal many changes between single and multiple generator type. Depending on the frequency and ear, the type designated usually remained constant from week-week over one year (for left and right ears n = 44). Single generator sites were consistently attributed to the left ear for 2 kHz, 4 kHz, 6 kHz (with two multiple site exceptions) and 8 kHz, and the right ear for 4 kHz and 6 kHz (with one multiple site exception). Whereas, multiple generator sites were never consistently attributed to either ear for any frequency. However, for two frequencies in the right ear, the type designated did vary: at 2 kHz (22 single and 22 multiple); and, at 8 kHz (25 single and 19 multiple). The observed changes were between linear and either flat-steep or diphasic shapes. Thus, although input-output function measurements of -10 dBSPL iso-criterion and slope appear stable irrespective of the time of testing, shape can fluctuate considerably.

3.6.2. EFFECT OF AGE

DPOAE input-output function -10 dBSPL iso-criteria at 2, 4, 6 and 8 kHz were found not to correlate with age in control subjects ($p > 0.05$; except at 4 kHz for left ears only, where $p < 0.02$). Scatter plots and regression analyses are shown, for all four frequencies in Figures 3.6.2.1., 3.6.2.2., 3.6.2.3. and 3.6.2.4. respectively. Similarly, DPOAE slope measurements did not correlate with age in control subjects ($p > 0.05$; except at 4 kHz for right ears only, where $p < 0.02$), as shown in Figures 3.6.2.5., 3.6.2.6., 3.6.2.7. and 3.6.2.8..

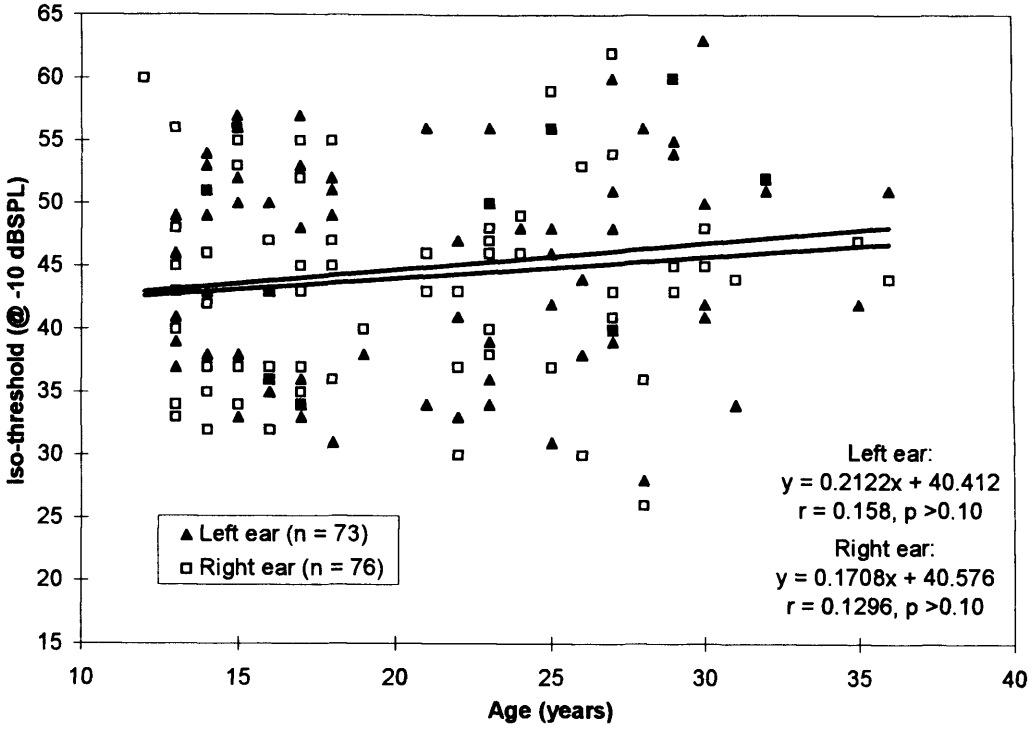


Fig. 3.6.2.1. 2 kHz DPOAE growth rate iso-thresholds: Effect of age in controls. Mean iso-threshold+/-SD for left ears = 44.90+/-8.55 dBSPL and right ears = 44.08+/-8.16 dBSPL.

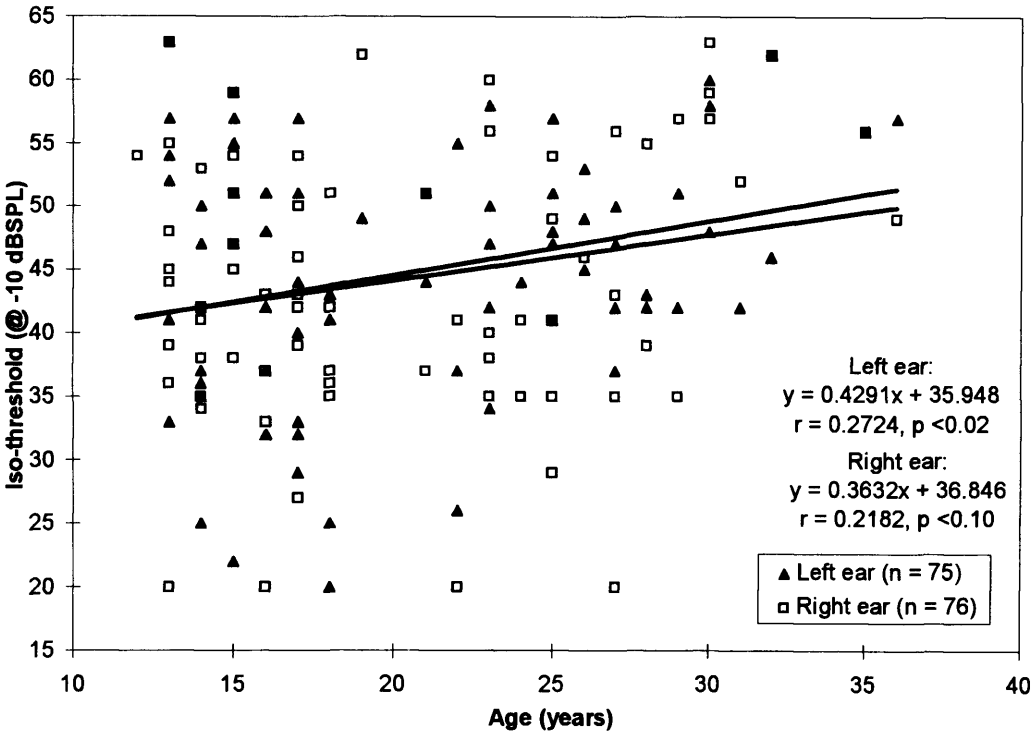


Fig. 3.6.2.2. 4 kHz DPOAE growth rate iso-thresholds: Effect of age in controls. Mean iso-threshold+/-SD for left ears = 44.87+/-9.86 dBSPL and for right ears = 44.22+/-10.49 dBSPL.

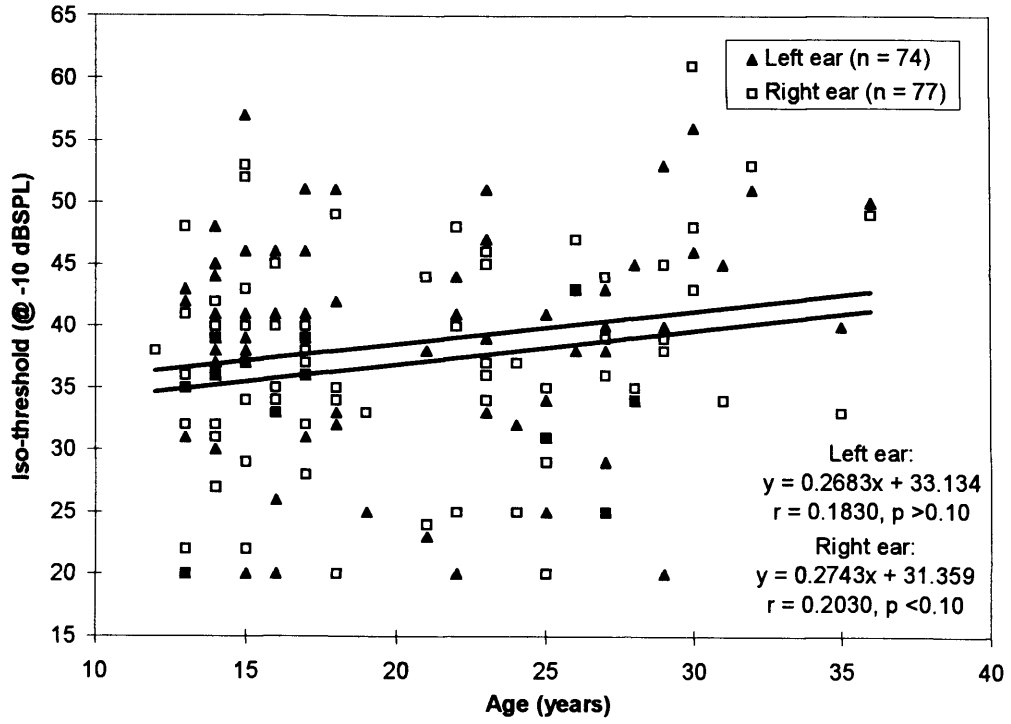


Fig. 3.6.2.3. 6 kHz DPOAE growth rate iso-thresholds: Effect of age in controls. Mean iso-threshold+/-SD for left ears = 38.65+/-9.09 dBSPL and right ears = 36.97+/-8.41 dBSPL.

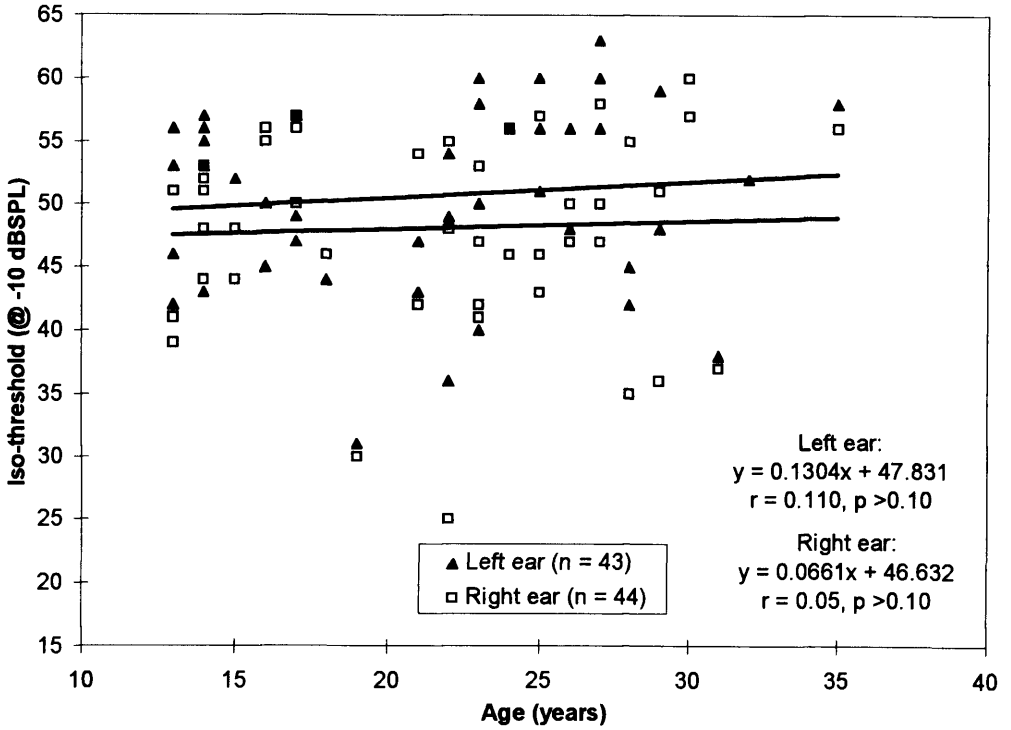


Fig. 3.6.2.4. 8 kHz DPOAE growth rate iso-thresholds: Effect of age in controls. Mean iso-threshold+/-SD for left ears = 50.61+/-7.28 dBSPL and right ears = 48.07+/-7.84 dBSPL.

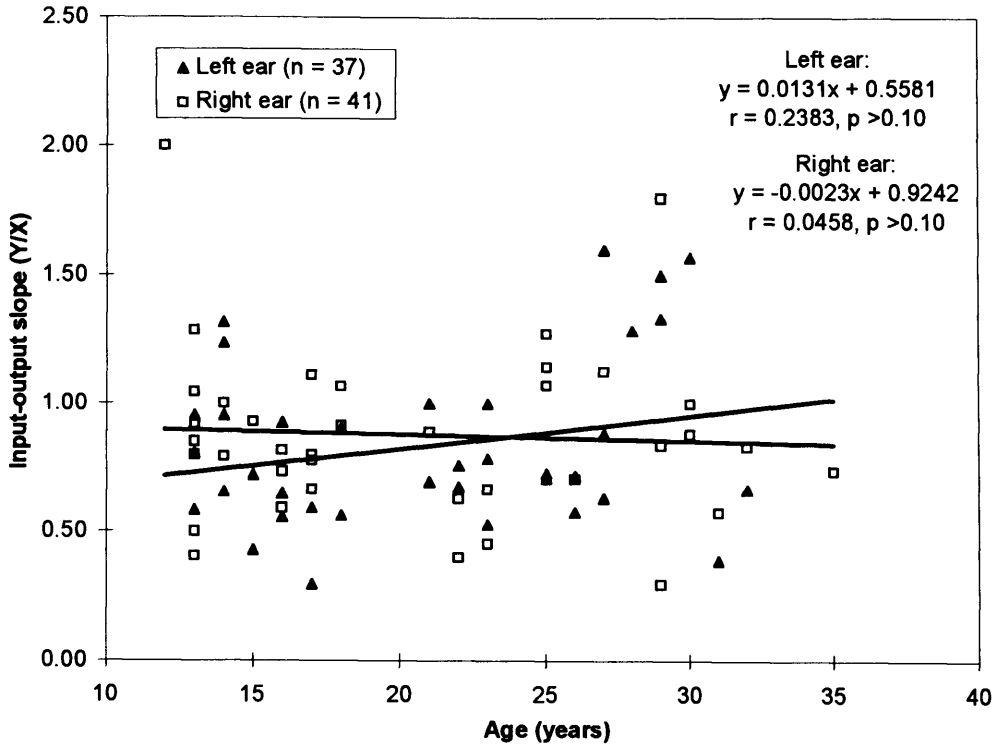


Fig. 3.6.2.5. 2 kHz DPOAE growth rate slopes: Effect of age in controls. Mean slope+/-SD for left ears = 0.84+/-0.33 and right ears = 0.88+/-0.33.

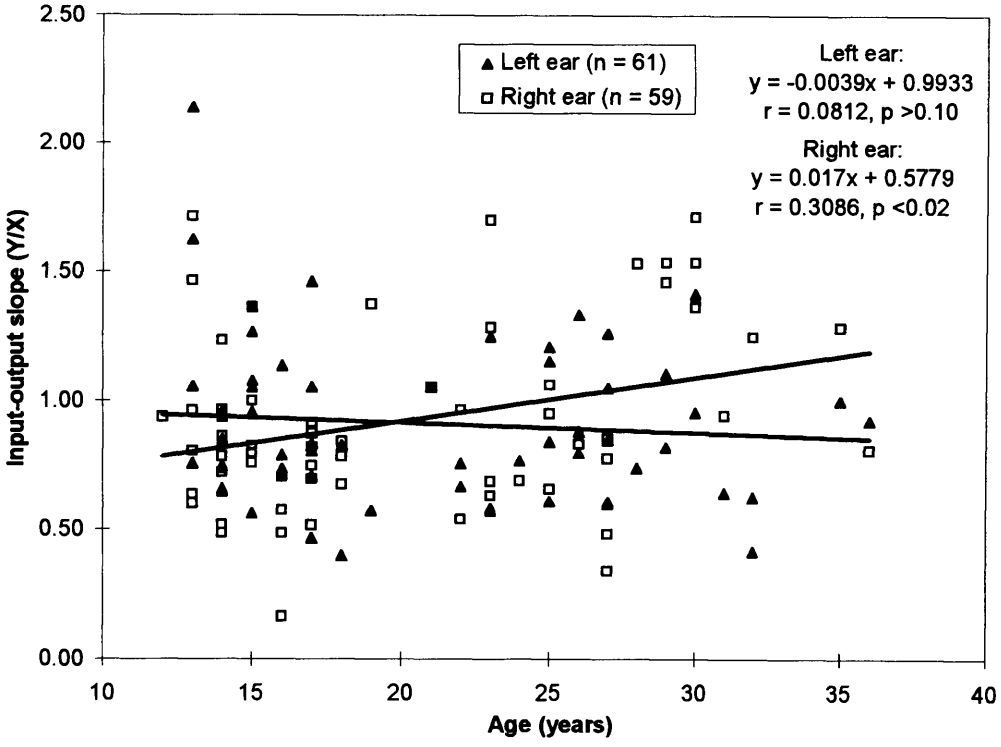


Fig. 3.6.2.6. 4 kHz DPOAE growth rate slopes: Effect of age in controls. Mean slope+/-SD for left ears = 0.91+/-0.32 and right ears = 0.93+/-0.36.

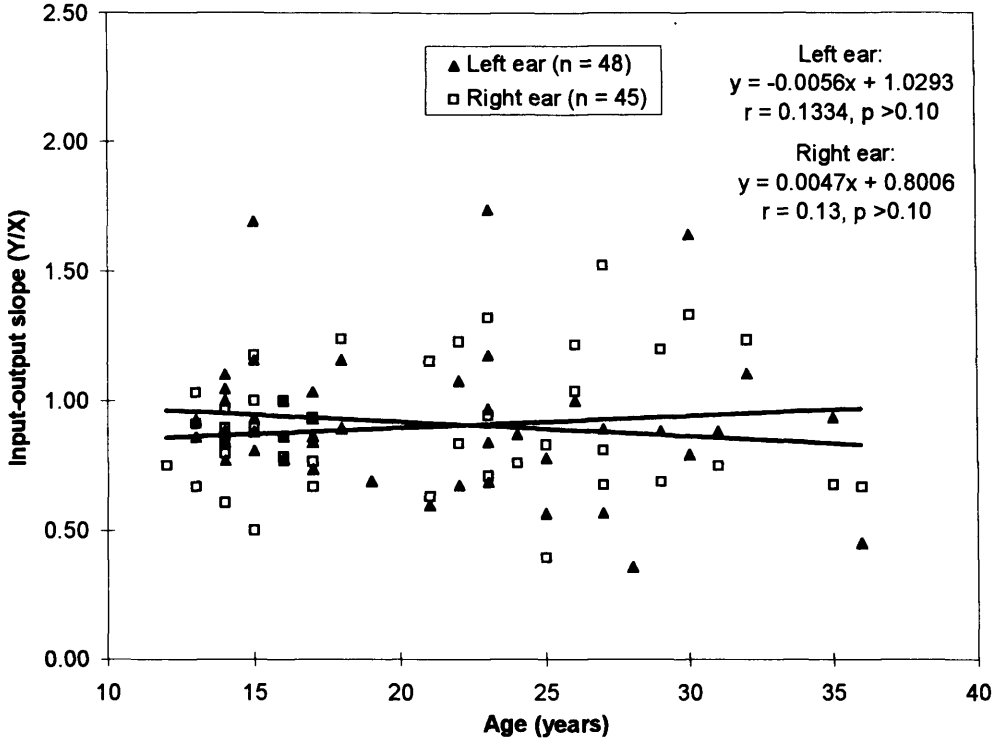


Fig. 3.6.2.7. 6 kHz DPOAE growth rate slopes: Effect of age in controls. Mean slope+/-SD for left ears = 0.91+/-0.27 and right ears = 0.90+/-0.24.

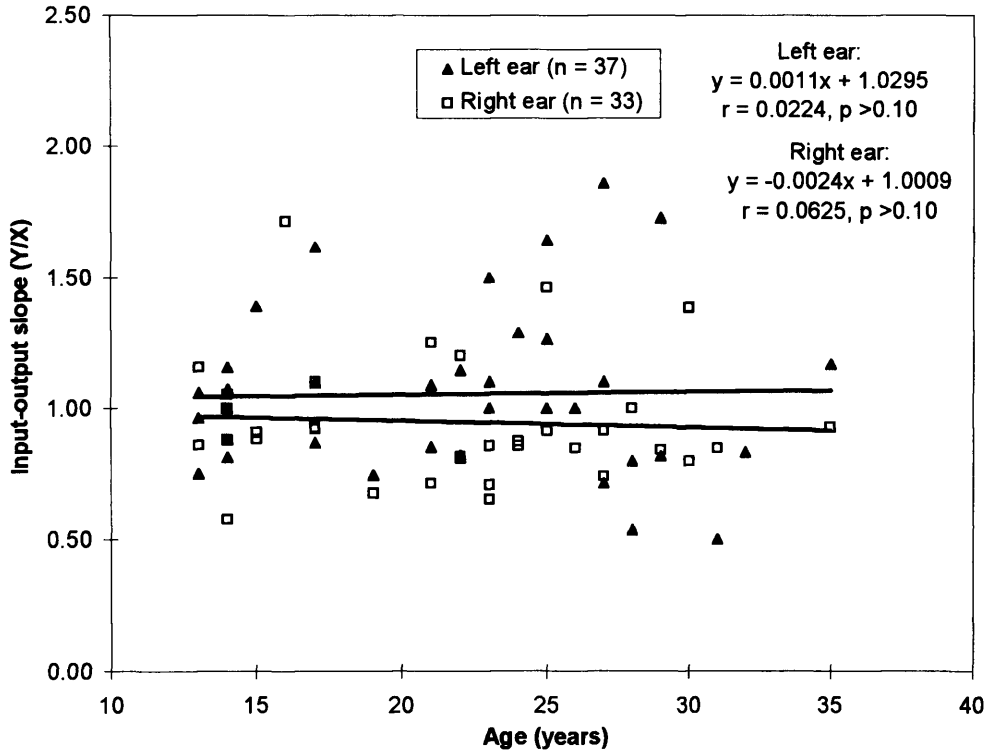


Fig. 3.6.2.8. 8 kHz DPOAE growth rate slopes: Effect of age in controls. Mean slope+/-SD for left ears = 1.05+/-0.31 and right ears = 0.95+/-0.24.

As input-output function shape is categorical, control subjects were split into two groups of approximately equal number (from 6-19 and 20-37 years old). For input-output function shapes at 2, 4, 6 and 8 kHz, there were no significant differences (χ^2, $p > 0.05$ in all cases) between juvenile and adult groups of control subjects (see Table 3.6.2.).

Frequency	Group	Left ear shape type Single / Multiple	Right ear shape type Single / Multiple	Combined left + right % Multiple
2 kHz	Juvenile	33 / 4	36 / 6	15
	Adult	31 / 6 $\chi^2 = 0.46$, $p = 0.497$	29 / 8 $\chi^2 = 0.73$, $p = 0.394$	23
4 kHz	Juvenile	36 / 5	35 / 8	18
	Adult	34 / 3 $\chi^2 = 0.35$, $p = 0.552$	30 / 6 $\chi^2 = 0.05$, $p = 0.822$	14
6 kHz	Juvenile	31 / 9	33 / 9	28
	Adult	28 / 7 $\chi^2 = 0.70$, $p = 0.792$	26 / 10 $\chi^2 = 0.42$, $p = 0.515$	32
8 kHz	Juvenile	19 / 3	18 / 3	16
	Adult	25 / 2 $\chi^2 = 0.51$, $p = 0.474$	24 / 6 $\chi^2 = 0.28$, $p = 0.598$	16

TABLE. 3.6.2. Number of single and multiple component DPOAE input-output function shapes at 2, 4, 6 and 8 kHz for juvenile and adult control subjects.

Therefore for all four frequencies, there was no consistent correlation ($p > 0.05$ for both left and right ears) of age with either -10 dBSPL iso-criterion or slope. Similarly, there were no significant differences ($p > 0.05$) in the number of generator sites (inferred by input-output function shape). Hence there was no need to partition groups according to age.

3.6.3. EFFECT OF DOSE

DPOAE input-output function -10 dBSPL iso-criteria at 2, 4, 6 and 8 kHz were found not to correlate with dose in patients ($p > 0.05$ in all cases). Dose-response scatter plots and regression analyses are shown, for all four frequencies in Figures 3.6.3.1., 3.6.3.2., 3.6.3.3. and 3.6.3.4. respectively. Similarly, DPOAE slope measurements did not correlate with dose in patients ($p > 0.05$ in all cases), as shown in Figures 3.6.3.5., 3.6.3.6., 3.6.3.7. and 3.6.3.8.. (In all figures, A. shows left ears and B. shows right ears.)

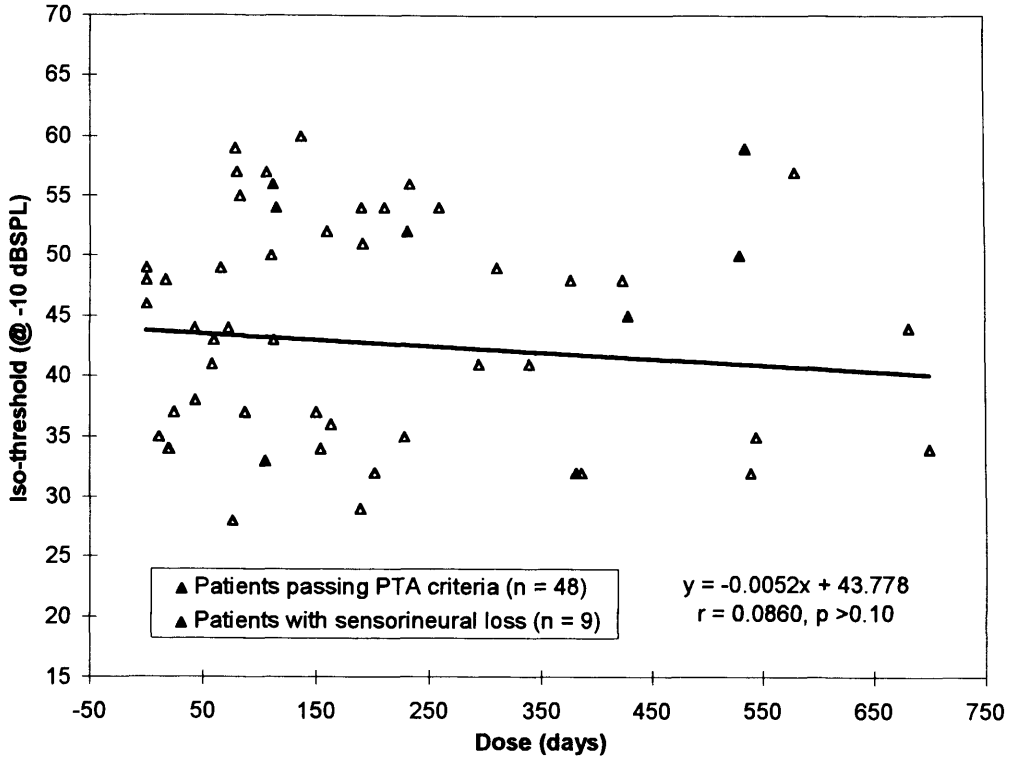


Fig. 3.6.3.1.A. 2 kHz DPOAE growth rate iso-thresholds: Dose-response for left ears. Mean iso-threshold+/-SD = 42.88+/-10.72 dBSPL (n = 48).

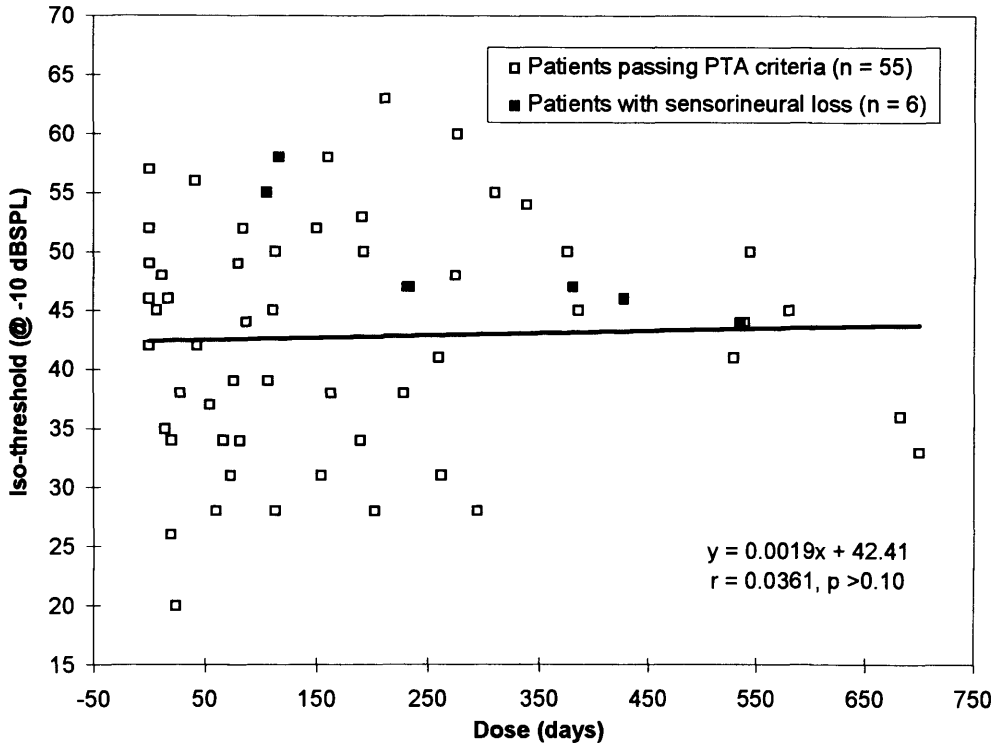


Fig. 3.6.3.1.B. 2 kHz DPOAE growth rate iso-threholds: Dose-response for right ears. Mean iso-threshold+/-SD = 42.75+/-9.79 dBSPL (n = 55).

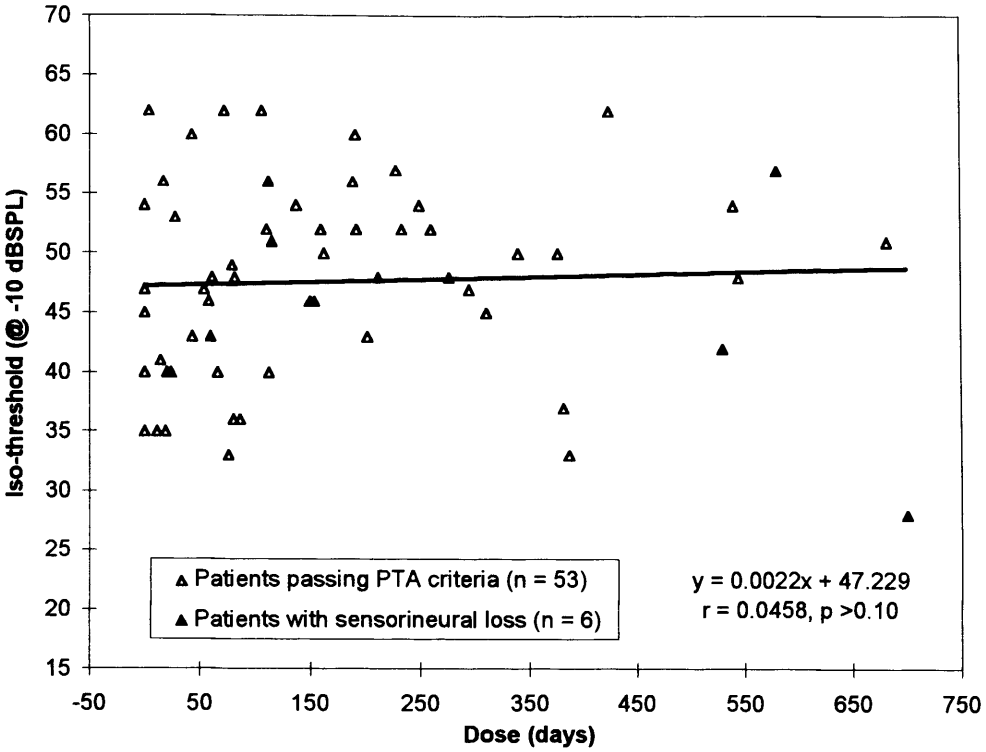


Fig. 3.6.3.2.A. 4 kHz DPOAE growth rate iso-thresholds: Dose-response for left ears. Mean iso-threshold+/-SD = 47.60+/-8.37 dBSPL (n = 53).

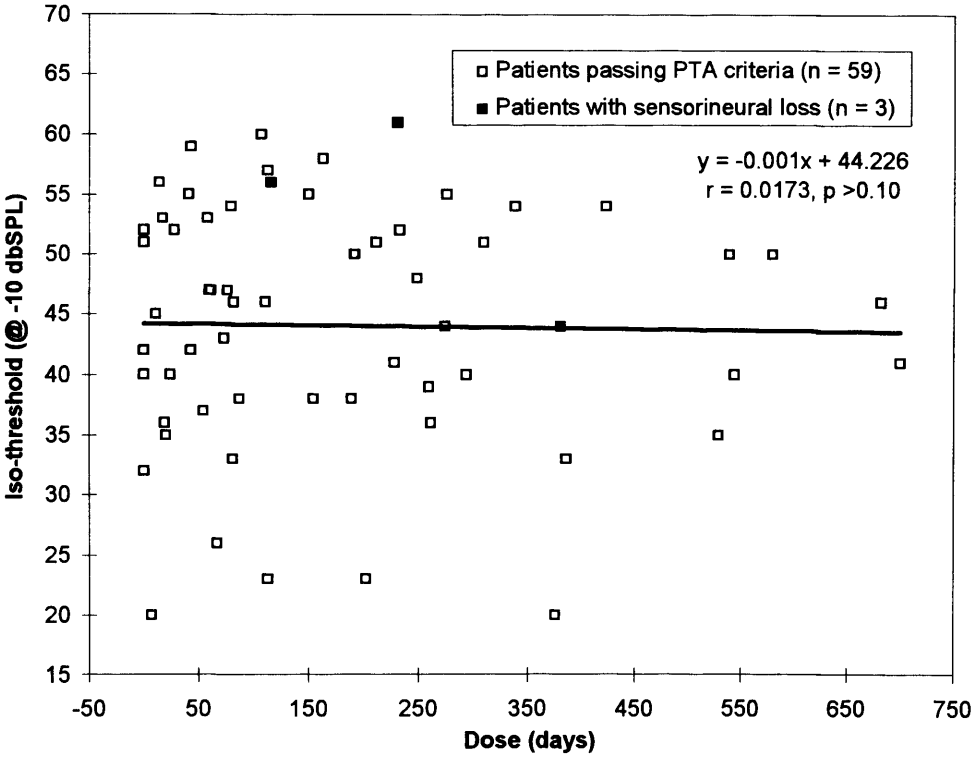


Fig. 3.6.3.2.B. 4 kHz DPOAE growth rate iso-thresholds: Dose-response for right ears. Mean iso-threshold+/-SD = 44.05+/-9.89 dBSPL (n = 59).

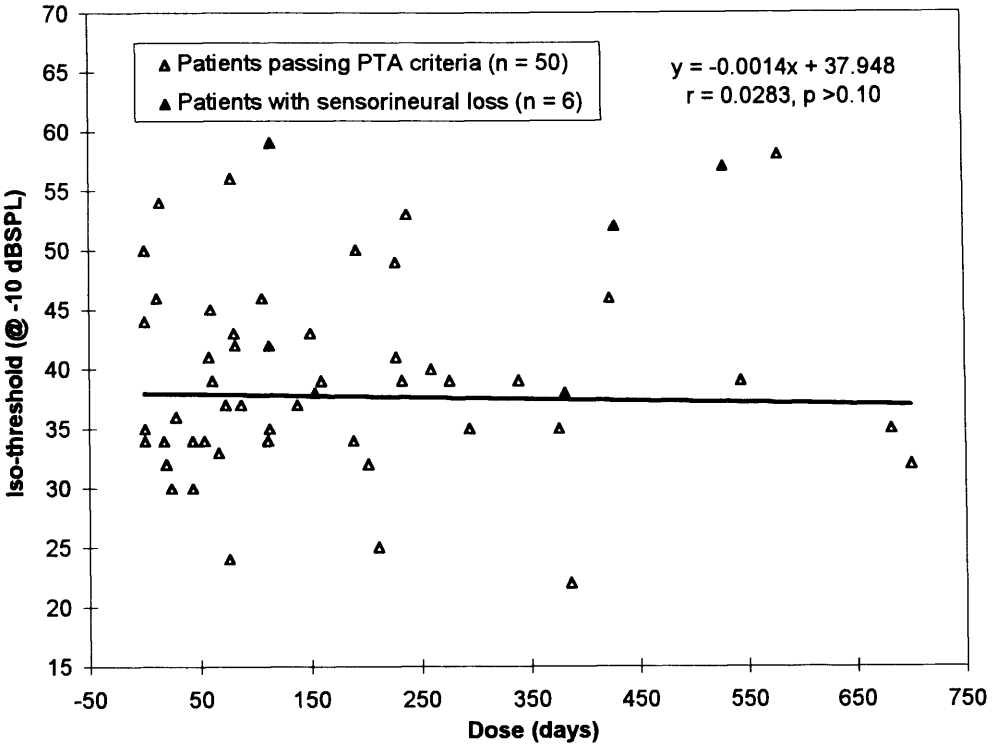


Fig. 3.6.3.3.A. 6 kHz DPOAE growth rate iso-thresholds: Dose-response for left ears. Mean iso-threshold+/-SD = 37.72+/-8.71 dBSPL (n = 50).

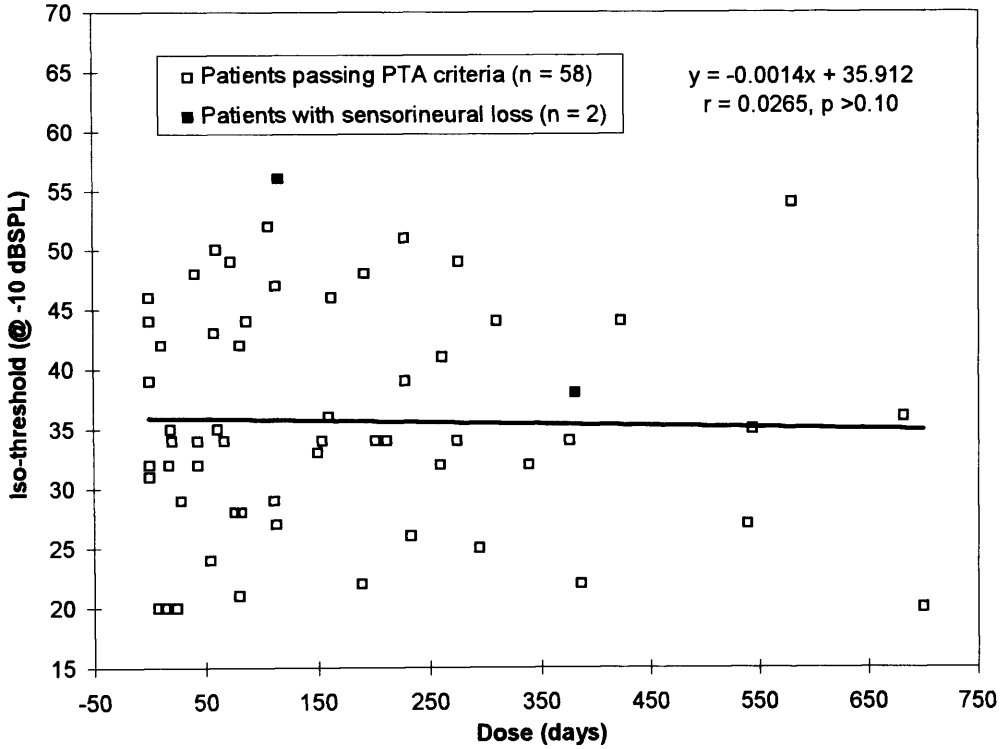


Fig. 3.6.3.3.B. 6 kHz DPOAE growth rate iso-thresholds: Dose-response for right ears. Mean iso-threshold+/-SD = 35.67+/-9.37 dBSPL (n = 58).

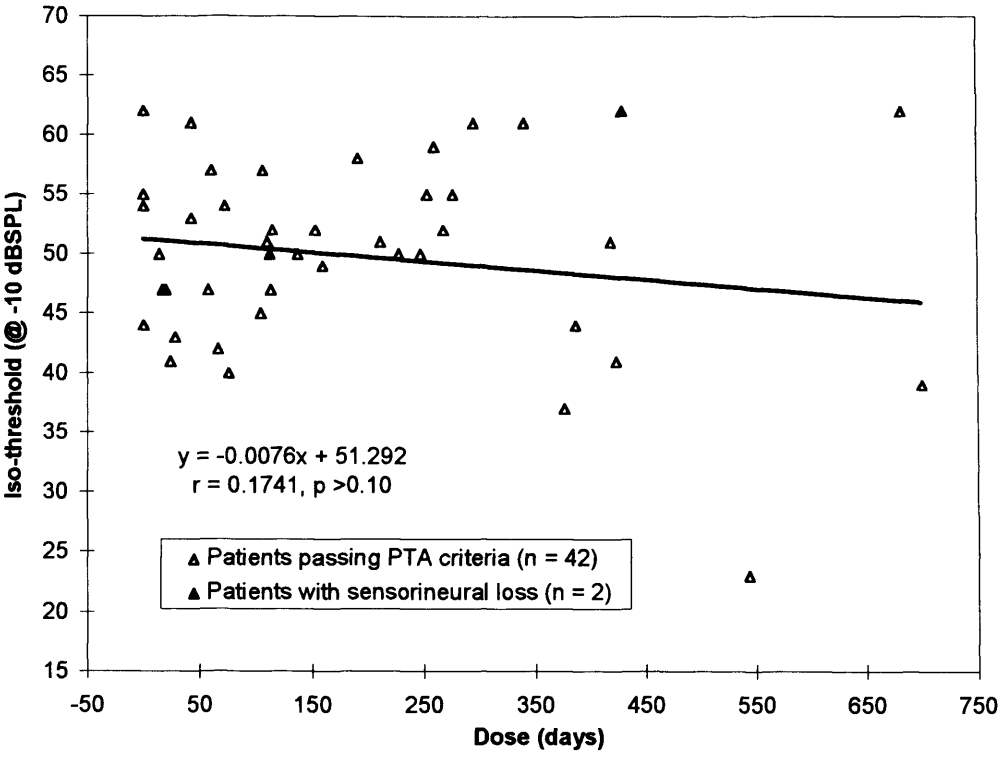


Fig. 3.6.3.4.A. 8 kHz DPOAE growth rate iso-thresholds: Dose-response for left ears. Mean iso-threshold+/-SD = 49.91+/-7.90 dBSPL (n = 42).

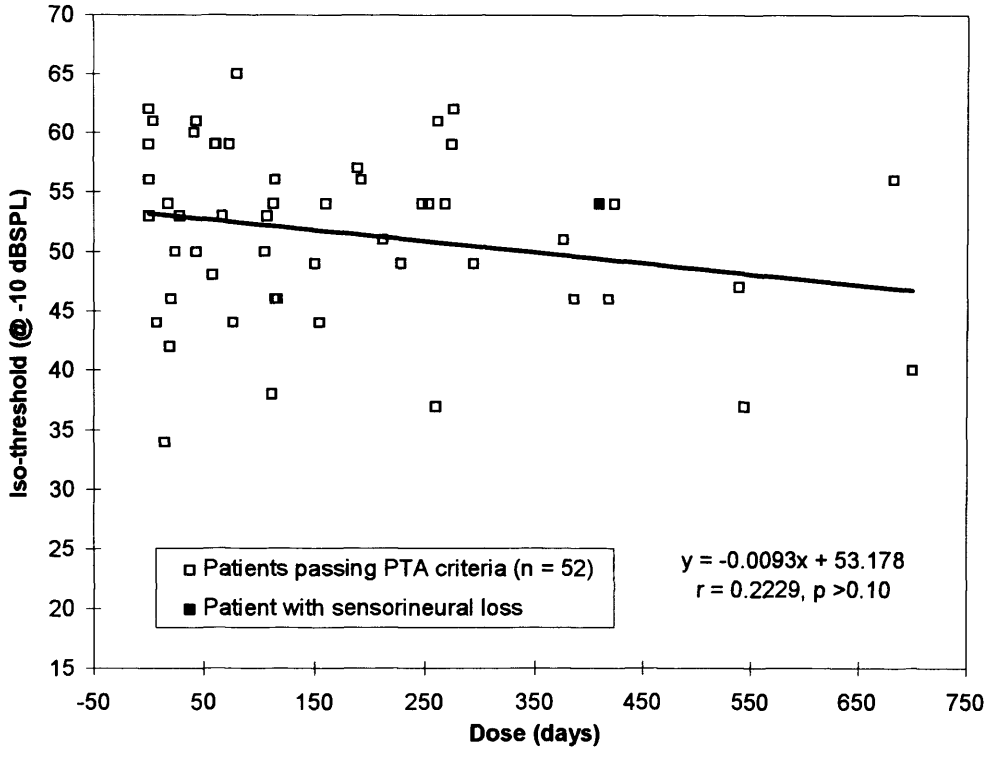


Fig. 3.6.3.4.B. 8 kHz DPOAE growth rate iso-thresholds: Dose-response for right ears. Mean iso-threshold+/-SD = 51.58+/-7.28 dBSPL (n = 52).

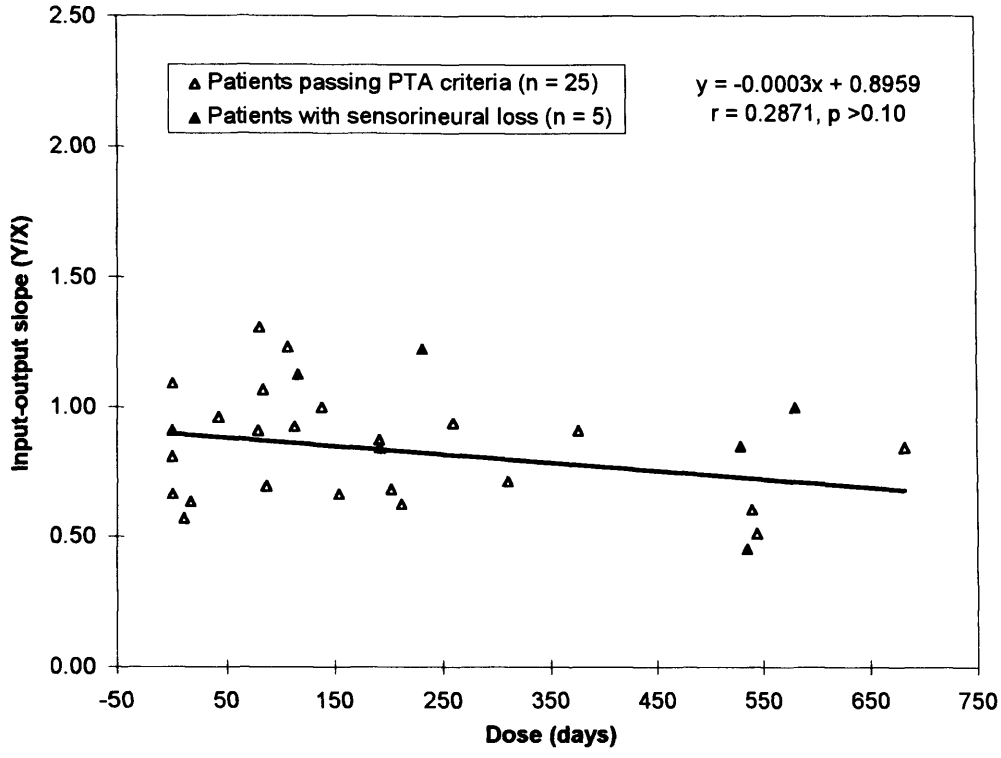


Fig. 3.6.3.5.A. 2 kHz DPOAE growth rate slopes: Dose-response for left ears. Mean slope+/-SD = 0.84+/-0.22 (n = 25).

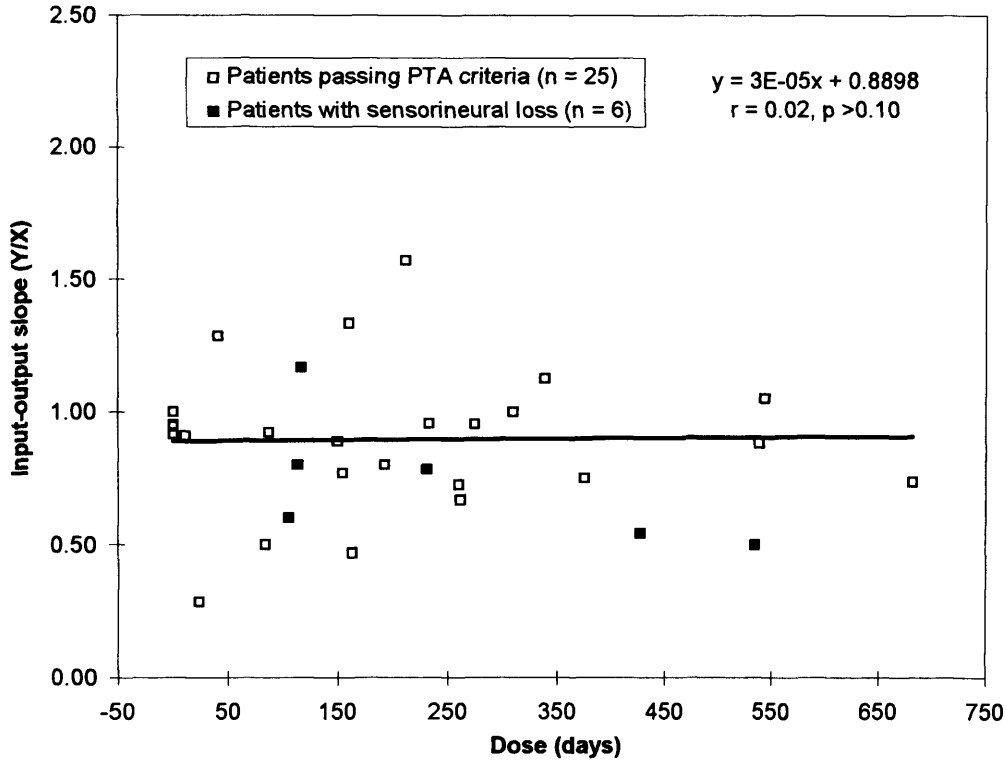


Fig. 3.6.3.5.B. 2 kHz DPOAE growth rate slopes: Dose-response for right ears. Mean slope+/-SD = 0.90+/-0.27 (n = 25).

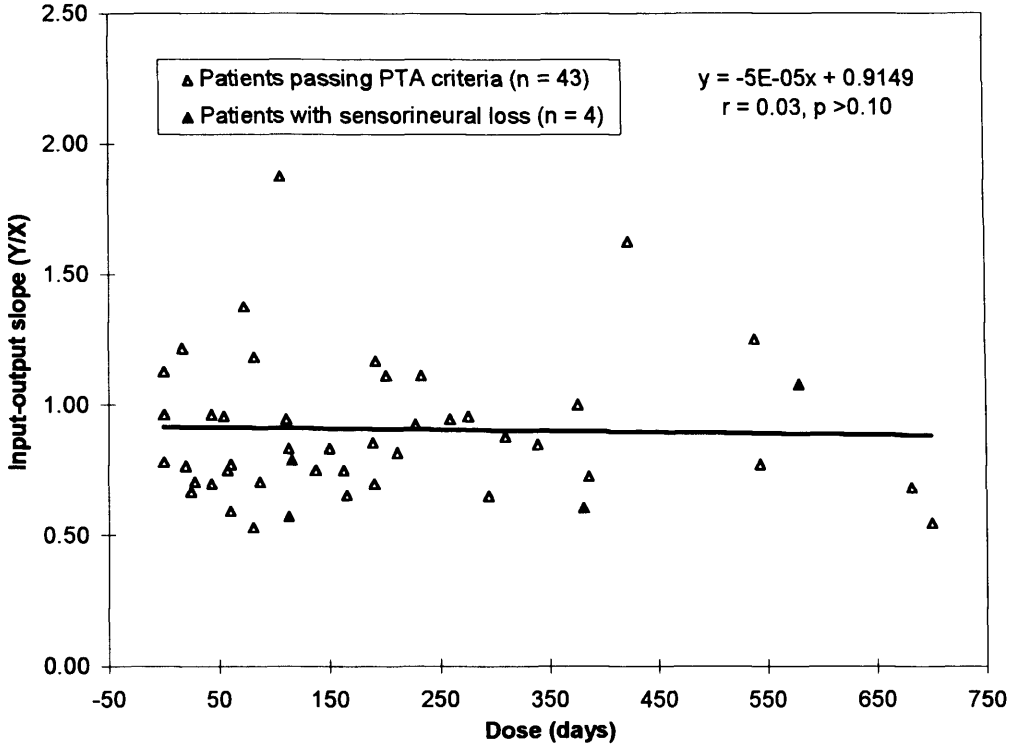


Fig. 3.6.3.6.A. 4 kHz DPOAE growth rate slopes: Dose-response for left ears. Mean slope+/-SD = 0.91+/-0.27 (n = 43).

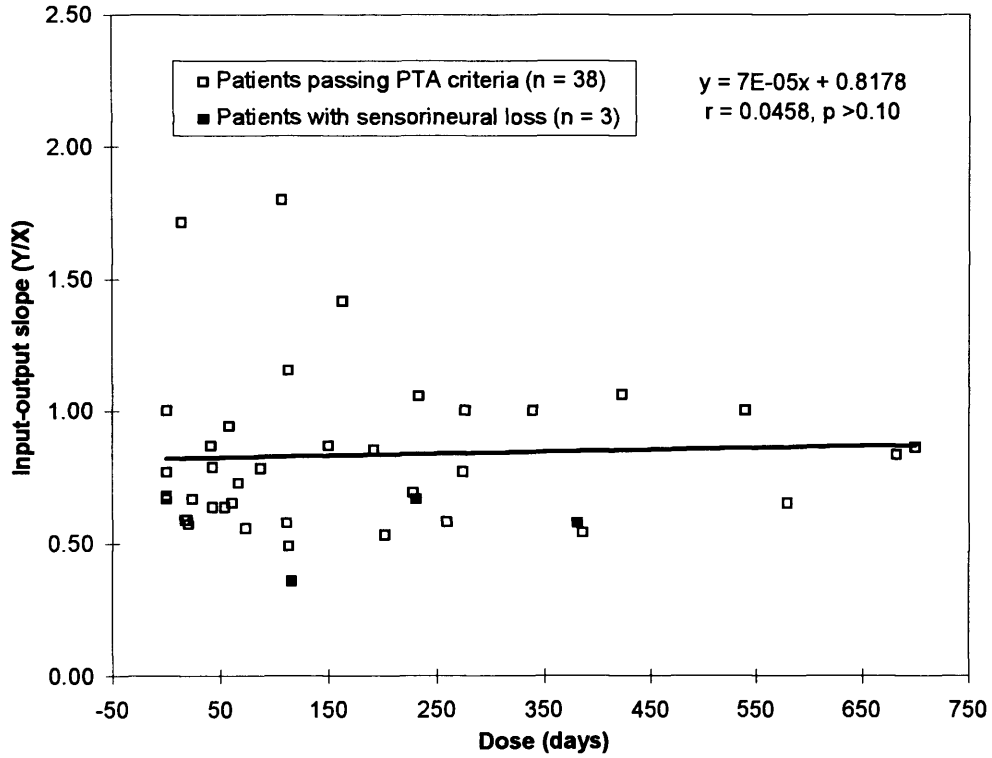


Fig. 3.6.3.6.B. 4 kHz growth rate slopes: Dose-response for right ears. Mean slope+/-SD = 0.83+/-0.31 (n = 38).

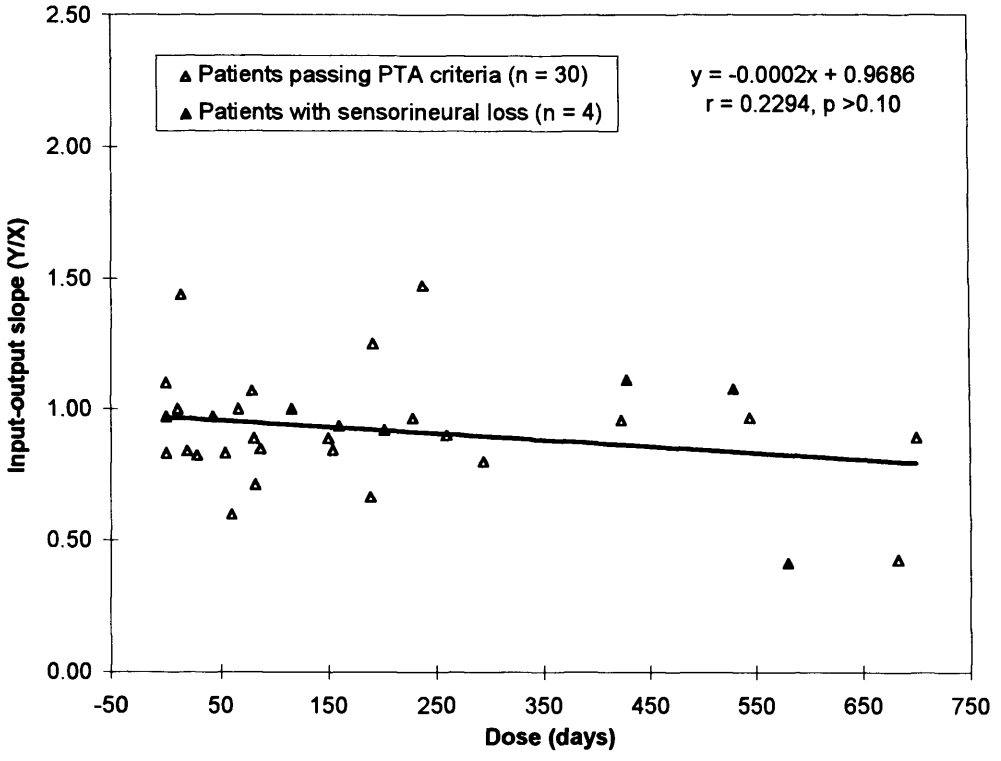


Fig. 3.6.3.7.A. 6 kHz DPOAE growth rate slopes: Dose-response for left ears. Mean slope+/-SD = 0.93+/-0.21 (n = 30).

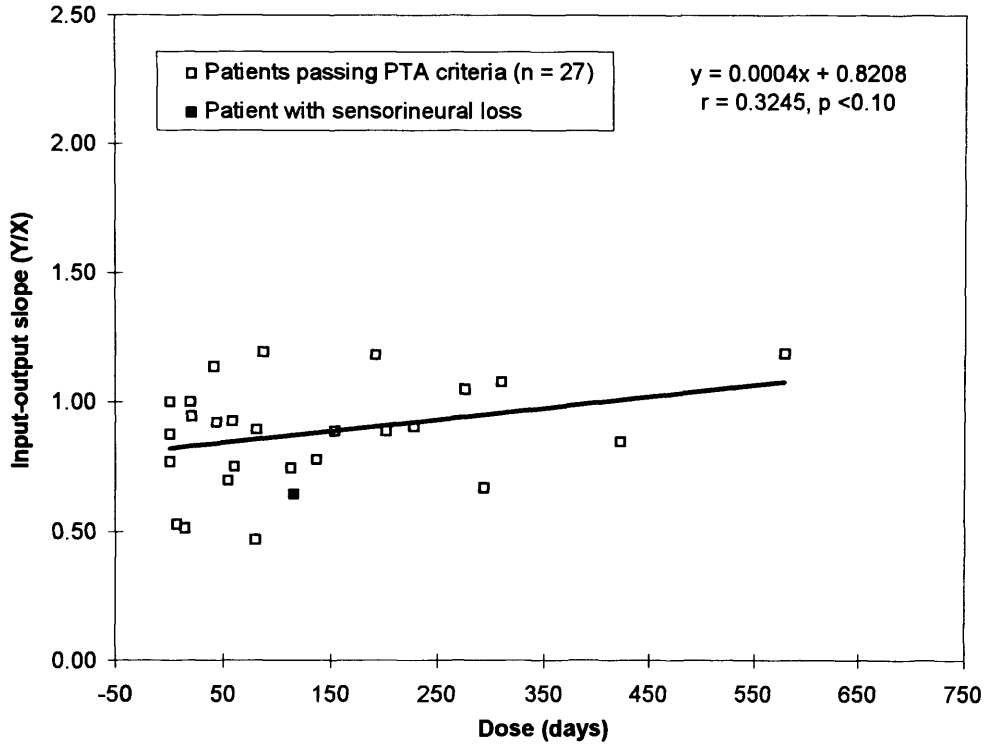


Fig. 3.6.3.7.B. 6 kHz DPOAE growth rate slopes: Dose-reponse for right ears. Mean slope+/-SD = 0.88+/-0.20 (n = 27).

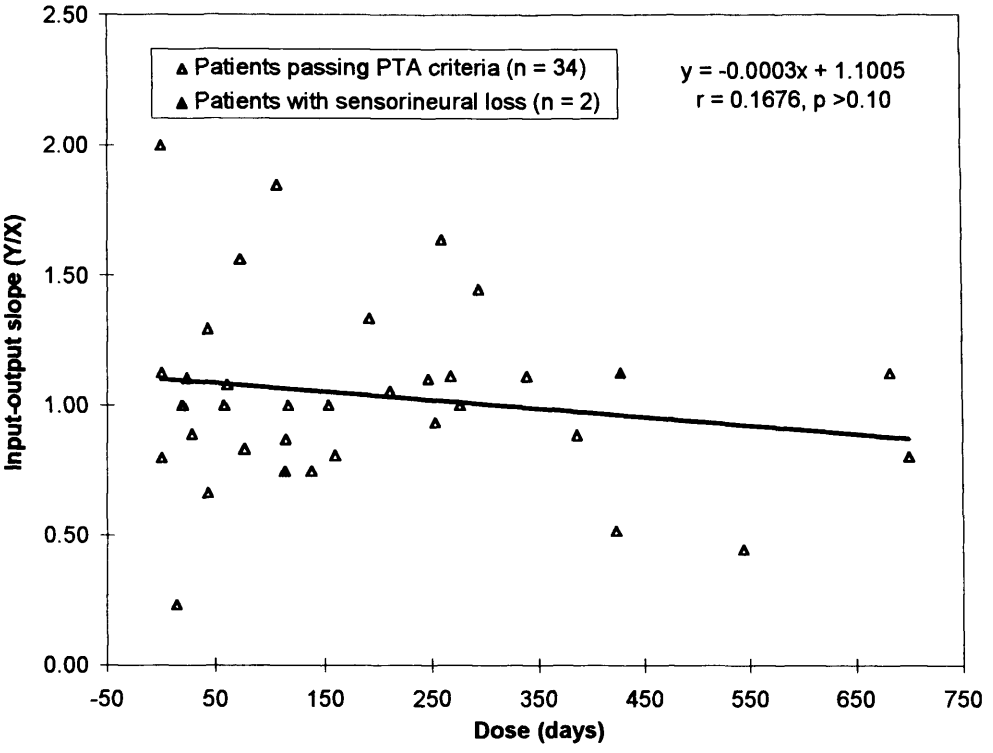


Fig. 3.6.3.8.A. 8 kHz DPOAE growth rate slopes: Dose-response for left ears. Mean slope+/-SD = 1.04+/-0.36 (n = 34).

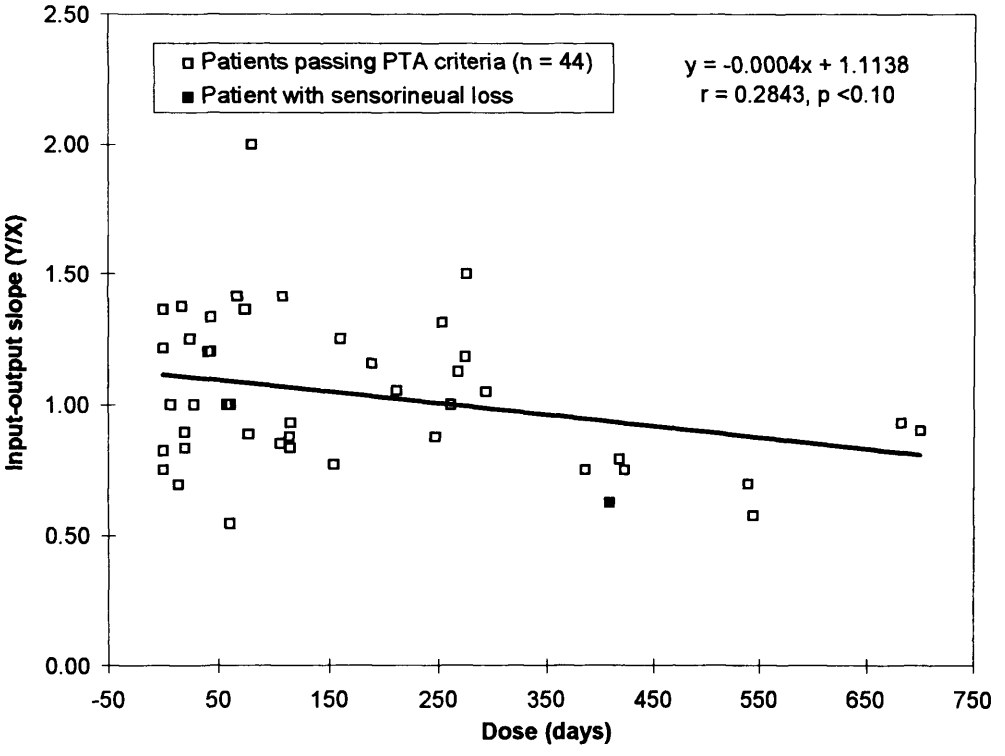


Fig. 3.6.3.8.B. 8 kHz DPOAE growth rate slopes: Dose-response for right ears. Mean slope+/-SD = 1.04+/-0.29 (n = 44).

As input-output function shape is categorical, patients were split into two groups of approximately equal number (from 0-99 and 100-700 days total exposure). For input-output function shapes at 2, 4, 6 and 8 kHz, there were no significant differences (χ^2, $p > 0.05$ in all cases) between low and high dose groups (see Table 3.6.3.).

Frequency	Group	Left ear shape type Single / Multiple	Right ear shape type Single / Multiple	Combined left + right % Multiple
2 kHz	Low dose	16 / 6	19 / 7	38
	High dose	20 / 7 $\chi^2 = 0.01$, $p = 0.915$	26 / 2 $\chi^2 = 3.80$, $p = 0.051$	20
4 kHz	Low dose	22 / 3	23 / 5	18
	High dose	25 / 2 $\chi^2 = 0.32$, $p = 0.575$	24 / 4 $\chi^2 = 0.13$, $p = 0.716$	12
6 kHz	Low dose	23 / 2	24 / 4	13
	High dose	25 / 0 $\chi^2 = 2.08$, $p = 0.149$	22 / 6 $\chi^2 = 0.49$, $p = 0.485$	13
8 kHz	Low dose	17 / 2	20 / 0	5
	High dose	27 / 1 $\chi^2 = 0.92$, $p = 0.339$	26 / 4 $\chi^2 = 2.90$, $p = 0.089$	9

TABLE. 3.6.3. Number of single and multiple component DPOAE input-output function shapes at 2, 4, 6 and 8 kHz for low and high dose patient groups.

Therefore for all four frequencies, there was no correlation ($p > 0.05$) of dose with either -10 dBSPL iso-criterion or slope. Furthermore, there was no obvious clustering of patients either exposed to high peak serum levels, or with sensorineural loss. Similarly, there were no significant differences ($p > 0.05$) in the number of generator sites (inferred by input-output function shape). Hence there was no need to partition groups according to dose.

3.6.4. CONTROLS VERSUS PATIENTS

For input-output function -10 dBSPL iso-criteria and slopes (at 2, 4, 6 and 8 kHz), comparison of control and patient groups revealed no significant differences ($p > 0.05$) even when including patients with bilateral sensorineural loss (see Tables 3.6.4.1. and 3.6.4.2. respectively). For input-output function shapes (at the same frequencies), comparison of control and patient groups revealed only two - unsupported* - significant differences ($p < 0.05$). For left ears only, at 2 kHz patients had a higher proportion of multiple generators ($\chi^2 = 4.52$, $df = 1$, $p = 0.034$), whereas at 6 kHz patients had a lower proportion of multiple generators ($\chi^2 = 8.45$, $df = 1$, $p = 0.004$). When including patients with bilateral sensorineural loss, no additional significant differences ($p > 0.05$) in shape were apparent (see Table 3.6.4.3.). In all cases, patients who had received no aminoglycoside exposure were excluded.

* Due to the multiple comparison design, a biologically plausible difference was considered to require a significant change in either both ears or adjacent frequencies (or both). (i.e. with $p > 0.05$ a statistically significant difference will occur by chance in 1 out of 20 comparisons.)

Frequency	Group	Left ear iso-criterion Mean+/-SD (n) in dBSPL	Right ear iso-criterion Mean+/-SD (n) in dBSPL
2 kHz	Controls	44.90+/-8.55 (73)	44.08+/-8.16 (76)
	Patients without loss	42.35+/-11.21 (43) Mann-Whitney, p=0.277	41.67+/-10.15 (46) Type II T, p=0.153
	Patients + those with loss	42.78+/-11.16 (49) Mann-Whitney, p=0.254	42.58+/-10.02 (52) Type II T, p=0.353
4 kHz	Controls	44.87+/-9.86 (75)	44.22+/-10.49 (76)
	Patients without loss	47.96+/-8.47 (48) Type II T, p=0.076	44.00+/-10.20 (50) Type II T, p=0.906
	Patients + those with loss	47.71+/-8.39 (51) Type II T, p=0.095	44.55+/-10.30 (53) Type II T, p=0.863
6 kHz	Controls	38.65+/-9.09 (74)	36.97+/-8.41 (77)
	Patients without loss	37.53+/-8.91 (45) Mann-Whitney, p=0.385	34.60+/-9.21 (50) Type II T, p=0.137
	Patients + those with loss	38.29+/-9.38 (48) Mann-Whitney, p=0.618	35.08+/-9.51 (52) Type II T, p=0.236
8 kHz	Controls	50.61+/-7.28 (43)	48.07+/-7.84 (44)
	Patients without loss	49.90+/-7.94 (38) Type II T, p=0.516	51.02+/-7.37 (47) Type II T, p=0.067
	Patients + those with loss	49.82+/-8.09 (39) Type II T, p=0.645	51.08+/-7.30 (49) Type II T, p=0.059

TABLE. 3.6.4.1. DPOAE input-output function -10 dBSPL iso-criteria (in dBSPL) at 2, 4, 6 and 8 kHz for control and patient groups.

Frequency	Group	Left ear slope Mean + /-SD (n)	Right ear slope Mean + /-SD (n)
2 kHz	Controls	0.84 + /-0.33 (37)	0.88 + /-0.33 (41)
	Patients without loss	0.84 + /-0.21 (21) Type III T, p = 0.994	0.89 + /-0.30 (21) Mann-Whitney, p = 0.715
	Patients + those with loss	0.85 + /-0.24 (24) Type II T, p = 0.879	0.85 + /-0.30 (26) Mann-Whitney, p = 0.908
4 kHz	Controls	0.91 + /-0.32 (61)	0.93 + /-0.36 (59)
	Patients without loss	0.90 + /-0.28 (40) Mann-Whitney, p = 0.945	0.83 + /-0.32 (32) Mann-Whitney, p = 0.203
	Patients + those with loss	0.89 + /-0.28 (42) Mann-Whitney, p = 0.806	0.81 + /-0.32 (35) Mann-Whitney, p = 0.088
6 kHz	Controls	0.91 + /-0.27 (48)	0.90 + /-0.24 (45)
	Patients without loss	0.92 + /-0.22 (26) Mann-Whitney, p = 0.683	0.86 + /-0.21 (22) Type II T, p = 0.578
	Patients + those with loss	0.93 + /-0.22 (28) Mann-Whitney, p = 0.484	0.85 + /-0.21 (23) Type II T, p = 0.467
8 kHz	Controls	1.05 + /-0.31 (37)	0.95 + /-0.24 (33)
	Patients without loss	1.01 + /-0.33 (31) Type II T, p = 0.621	1.04 + /-0.29 (39) Type II T, p = 0.141
	Patients + those with loss	1.02 + /-0.33 (32) Type II T, p = 0.123	1.03 + /-0.30 (40) Type II T, p = 0.191

TABLE. 3.6.4.2. DPOAE input-output function slopes (Y/X) at 2, 4, 6 and 8 kHz for control and patient groups.

Frequency	Group	Left ear shape type Single / Multiple	Right ear shape type Single / Multiple	Combined left+right % Multiple
2 kHz	Controls	64 / 10	65 / 14	19
	Patients without loss	31 / 13 $\chi^2=4.52$, $p=0.034$	40 / 8 $\chi^2=0.02$, $p=0.879$	30
	Patients + those with loss	35/ 15 $\chi^2=5.03$, $p=0.025$	45 / 9 $\chi^2=0.03$, $p=0.875$	30
4 kHz	Controls	70 / 8	65 / 14	16
	Patients without loss	44 / 3 $\chi^2=0.55$, $p=0.459$	42 / 8 $\chi^2=0.06$, $p=0.800$	13
	Patients + those with loss	50 / 3 $\chi^2=0.87$, $p=0.352$	48 / 8 $\chi^2=0.28$, $p=0.594$	11
6 kHz	Controls	59 / 16	59 / 19	32
	Patients without loss	44 / 1 $\chi^2=8.45$, $p=0.004$	41 / 9 $\chi^2=0.72$, $p=0.396$	12
	Patients + those with loss	49 / 2 $\chi^2=7.52$, $p=0.006$	47 / 9 $\chi^2=1.36$, $p=0.245$	12
8 kHz	Controls	44 / 5	42 / 9	16
	Patients without loss	41 / 2 $\chi^2=1.01$, $p=0.316$	43 / 4 $\chi^2=1.78$, $p=0.183$	7
	Patients + those with loss	45 / 2 $\chi^2=1.26$, $p=0.262$	47 / 4 $\chi^2=2.20$, $p=0.138$	7

TABLE. 3.6.4.3. Number of single and multiple component DPOAE input-output function shapes at 2, 4, 6 and 8 kHz for control and patient groups.

There were two additional observations which are of interest. Firstly, patients with bilateral sensorineural loss often had no measurable input-output function at the higher test frequencies (none out of 12 ears at 2 kHz, 6 out of 12 ears at both 4 and 6 kHz, and 5 out of 8 ears at 8 kHz). Secondly, with increasing frequency, patients had a higher proportion of saturating input-output functions when compared with control subjects (equal at 2 kHz, two-fold more at 4 kHz, three-fold more at 6 kHz, and four-fold more at 8 kHz; see Table. 3.6.4.4.).

Retrospective power analysis for -10 dBSPL iso-criterion reveals that with a mean standard deviation of 7.64 dB (pooling control left and right ears over all four frequencies), for 80% power and $p = 0.05$: to detect a difference of 4 dB between means, each group needed to include 57 measures; and to detect a difference of 5 dB between means, each group needed to include 37 measures. The actual number of patient measures (38-51 left, and 46-53 right ears; depending on hypothesis) and control measures (43-75 left and 44-77 right ears; depending on hypothesis) can therefore be expected to have detected a significant difference of 5 dB (at $p = 0.05$) in 80 % of cases.

Similarly, power analysis for slope reveals that with a mean standard deviation of 0.30 (pooling control left and right ears over all four frequencies): to detect a difference of 0.25 between means, each group needed to include 23 measures; and to detect a difference of 0.20 between means, each group needed to include 35 measures. The actual number of patient measures (21-42 left, and 21-40 right ears; depending on hypothesis) and control measures (37-61 left and 33-59 right ears; depending on hypothesis) can therefore be expected to have detected a significant difference of 0.2 (at $p = 0.05$) in 80 % of cases.

Hence, for all four frequencies, sample size was sufficient to detect a difference between patient and control groups of 5 dB in iso-criterion and 0.2 in slope. Inter-supporting significant differences in input-output function iso-criterion, slope and shape were not apparent, even when including patients with bilateral sensorineural loss.

Frequency	Group (ears)	Linear (%)	Saturating (%)	Diphasic / Non-monotonic (%)	Flat-steep (%)	None (%)	Passive (%)
2 kHz	Controls (153)	50	29	12	4	2	3
	Patients without loss (92)	46	27 $\chi^2 = 0.071$ $p = 0.79$	17	5	3	1
	Patients + those with loss (104)	48	25 $\chi^2 = 0.44$ $p = 0.51$	17	6	3	1
4 kHz	Controls (157)	76	6	11	3	1	4
	Patients without loss (97)	73	13 $\chi^2 = 4.46$ $p = 0.035$	7	4	0	2
	Patients + those with loss (109)	70	12 $\chi^2 = 3.25$ $p = 0.071$	6	4	6	3
6 kHz	Controls (153)	61	14	10	12	0	1
	Patients without loss (95)	49	40 $\chi^2 = 22.1$ $p = 2 \times 10^{-6}$	2	8	0	0
	Patients + those with loss (107)	47	36 $\chi^2 = 18.3$ $p = 2 \times 10^{-5}$	2	8	6	1
8 kHz	Controls (100)	69	2	6	8	10	5
	Patients without loss (90)	78	9 $\chi^2 = 4.51$ $p = 0.034$	1	6	0	7
	Patients + those with loss (98)	73	8 $\chi^2 = 3.92$ $p = 0.048$	1	5	5	7

TABLE. 3.6.4.4. Percentages of different DPOAE input-output function shapes observed at 2, 4, 6 and 8 kHz for control and patient groups: Here left and right ears are combined, in order to observe any overall trend in the proportion of different shapes.

3.7. OTO-ACOUSTIC EMISSIONS - SOAES

Only peaks at or above both 1 kHz and 0 dBSPL were included as SOAEs. Peaks occurring at the same frequency in both ears - particularly when close to the exclusion limits - were not included as SOAEs, as these may have been attributable to environmental noise. Peaks were designated manually +/-1 dB and +/- 10 Hz. Each peak had to be at or above 0 dBSPL and 6 dB or more above surrounding background noise (+/-50 Hz) to be designated as an SOAE. A recording was considered 'noisy' and excluded if the amplitude was continuously above -5 dBSPL for an octave or more, or above 0 dBSPL for 100 Hz or more.* The lowest recorded intensity was usually -16 dBSPL. Values were recorded below -16 dBSPL (most commonly -370.7 dBSPL), but these were obviously equipment artefact. Therefore, values below -16 dBSPL were moderated upwards to -16 dBSPL - rather than include without change or exclude - so as not to seriously bias calculations. For an example of SOAE recording see Figure 3.9.4.1. later, where a graphical comparison with CSSOAEs facilitates interpretation.

* In addition, breathing-in was observed to result in less noise than breathing out, therefore most of the data was collected when breathing in (especially when noise fluctuates around the rejection level).

3.7.1. TEST RE-TEST VARIABILITY

First of all, it was necessary to establish the number of averages (of sequential Fourier transformations) needed to maintain a consistent signal to noise ratio with each SOAE measurement. Figure 3.7.1.1. shows the effect of different numbers of averages on SOAE amplitude (for repeated recordings of the same SOAEs in one individual*). The amplitude of SOAE peaks was stable after an initial count of 10-30. Whereas, the amplitude of contralateral noise (at the same frequency as peaks) decays exponentially with increasing number of averages. The number of sequential counts needed before reaching a steady state was observed to be between 100 and 250.

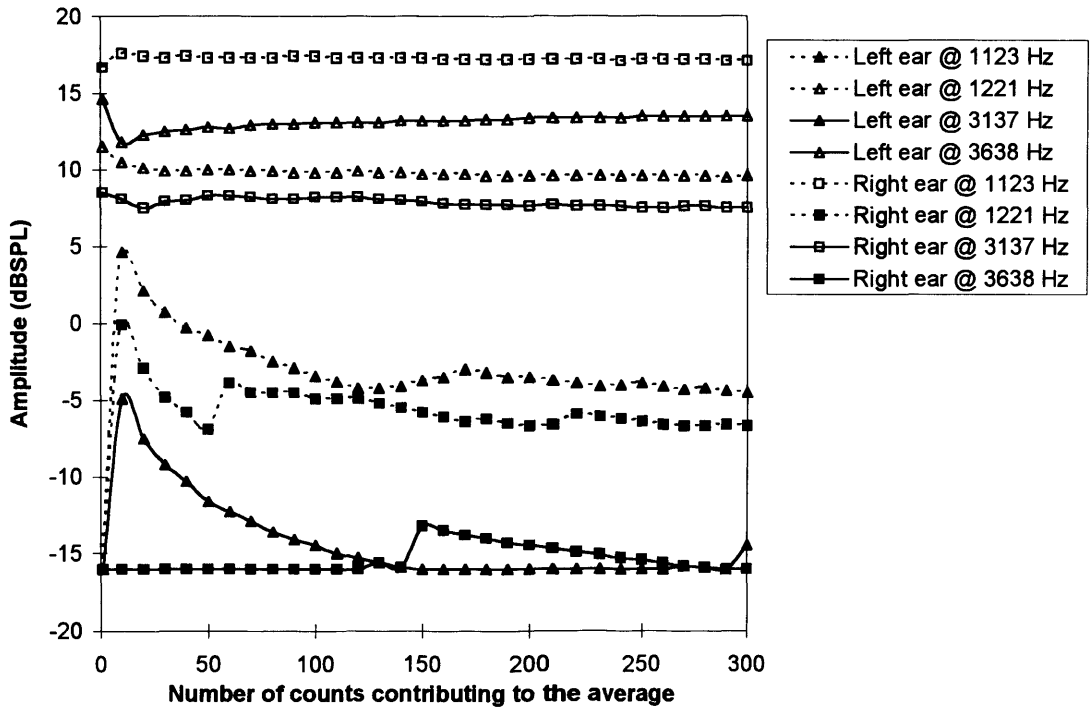


Fig. 3.7.1.1. Improvement of SOAE signal to noise with increasing data collection. The top four lines represent SOAE amplitudes, whereas the bottom four lines represent the corresponding noise amplitudes measured in the contralateral ear

* Peaks repeated within +/-12.5 Hz were assumed to be variation of the same SOAE.

For SOAE amplitude (dBSPL), repeated measurements in one representative control individual were found to be approximately equally stable, irrespective of the time of testing (see Table 3.7.1.). With increasing interval between measurements the amplitude variance increases for all assessed SOAEs regardless of absolute amplitude or frequency. Overall, the absolute amplitude measurements of the same SOAE appear stable irrespective of the frequency or ear. However, for all minute-minute measurements of the 3.137 kHz SOAE in the right ear the absolute amplitude was consistently lower than either AM-PM or week-week measurements. Thus, minute-minute measurements of SOAE amplitude appear more stable - although not necessarily representative - suggesting that AM-PM measurements would be a better indicator of long term reproducibility.

Ear	Frequency (kHz)	Minute-Minute Mean+/-SD (dBSPL)	AM-PM Mean+/-SD (dBSPL)	Week-Week Mean+/-SD (dBSPL)
Left	1.233	9.60+/-0.17	10.95+/-0.70	10.01+/-0.88
	3.650	14.14+/-0.18	12.57+/-.090	11.84+/-0.93
Right	1.123	17.67+/-0.29	16.34+/-0.74	16.39+/-0.94
	3.137	2.07+/-1.33	12.27+/-0.70	13.17+/-1.17

TABLE. 3.7.1. Test-retest variability of SOAE amplitude (dBSPL): Minute-minute over ten minutes (n = 10 sequential runs, after 150+/-2 averages), AM-PM over one week (n = 5 days AM and PM, after 260+/-2 averages) and week-week over one year (n = 47 weeks, after 150+/-2 averages).

Finally, Figure 3.7.1.2. shows the 6th order polynomial regression analyses of mean spectra for repeated measurements in one individual who had no SOAEs. Mean variance was found not to vary from week-week over one year (mean SD+/-SD: left ear = 1.53+/-1.19 dB; right ear = 1.26+/-1.09 dB; after 150+/-2 averages; n = 45 weeks and, 430 frequency points between 1001 and 6238 Hz inclusive). Thus, ear-canal attenuated background noise measurements appear stable irrespective of the time of testing.

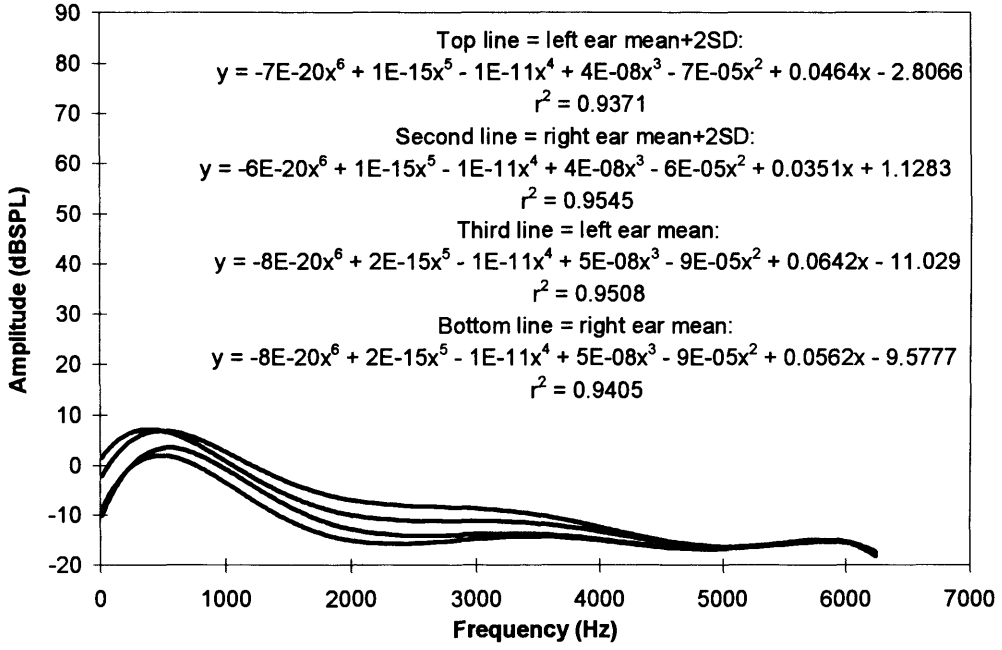


Fig. 3.7.1.2. Polynomial regression (6th order) of mean SOAE spectra for weekly repeatability

3.7.2. GENDER AND AURAL ASYMMETRIES

For analysis of SOAE occurrence, frequency and amplitude, control subjects were separated according to sex (female or male) and ear (left or right).

Figure 3.7.2. shows a scatter plot of amplitude versus frequency, summarising the relationship between females (n = 31 for both left and right ears) and males (n = 34 left ears, and 35 right ears). There were significantly more left ears with SOAEs than without SOAEs in females when compared with males (12 female and 3 male left ears had peaks; χ^2 = 8.16, df = 1, p = 0.004). There were no other significant findings (χ^2, p >0.05) concerning the number of ears with SOAEs (8 female and 4 male right ears had peaks). Overall, there were one or more SOAEs in: 39 % of female left ears; 26 % of female right ears; 9 % of male left ears; and, 11 % of male right ears. The maximum number of SOAEs was similar for all groups: female left ears = 3; female right ears = 2; male left ears = 4; and, male right ears = 3 peaks per ear. There were more peaks in female than male ears, but no more peaks in left ears than right ears, (SOAEs in: female left ears = 18; female right ears = 14; male left ears = 6; and, male right ears = 9). Overall, there were twice as many female than male SOAEs, and approximately equal numbers of left and right ear SOAEs. As there was evidently only an effect of gender on SOAEs, further analyses of SOAE occurrence were separated into female and male groups.

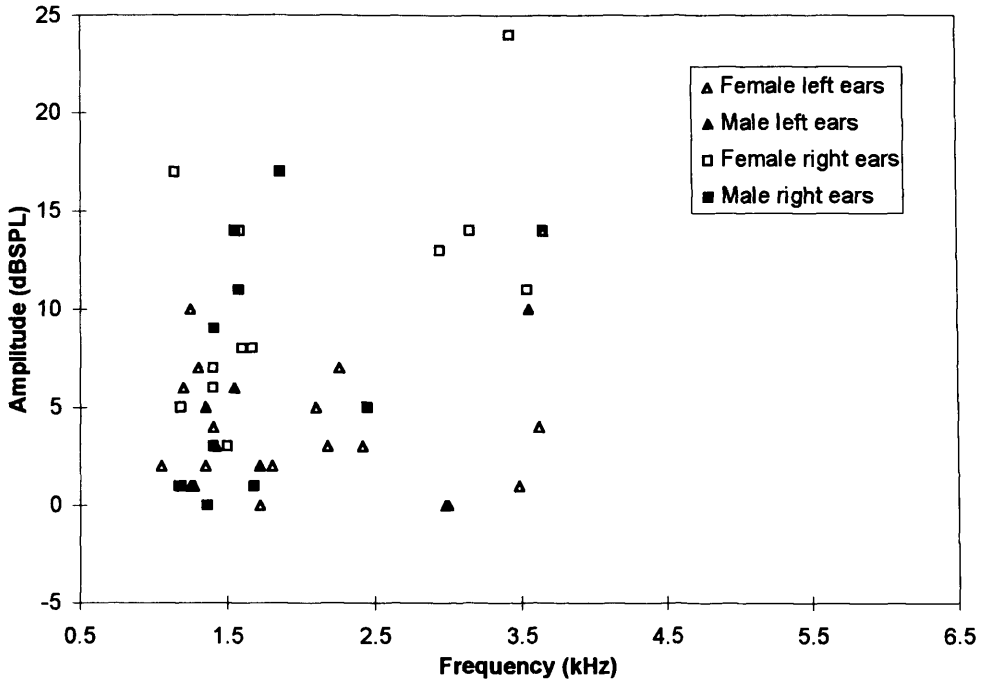


Fig. 3.7.2. Distribution of SOAEs in male and female controls

The frequency of female left ear SOAEs (mean+/-SD = 2.03+/-0.88 kHz), female right ear SOAEs (2.1+/-0.99 kHz), male left ear SOAEs (2.07+/-0.96 kHz), and male right ear SOAEs (1.61+/-0.37 kHz) were not significantly different ($p > 0.10$, all Type II T tests, except for Type III comparison of left and right in males). The maximum SOAE frequency was similar for all groups: female left ears = 3.65 kHz; female right ears = 3.65 kHz; male left ears = 3.55 kHz; and, male right ears = 2.45 kHz.

The maximum SOAE amplitude was similar for all groups: female left ears = 14 dBSPL; female right ears = 24 dBSPL; male left ears = 10 dBSPL; and, male right ears = 17 dBSPL. The amplitude of female left ear SOAEs (mean+/-SD = 4.11+/-3.63 dBSPL) was significantly lower than female right ear SOAEs (10.4+/-6.13 dBSPL; Type III T test, $p = 0.003$). However, there were no other significant findings ($p > 0.10$, all Type II T tests) concerning amplitude (mean+/-SD: male left ears = 4+/-3.74 dBSPL; and male right ears = 6.78+/-6.22 dBSPL).

For SOAE frequency (Hz) there were no significant differences ($p > 0.05$) with gender or ear. Furthermore, there were approximately equal

numbers of left and right ears with SOAEs (and equal numbers of SOAEs in left and right ears). However, the amplitude of SOAEs was consistently lower in left ears compared with right ears. Thus it was essential to ensure equal proportions of both ears in groups which were to be compared. Furthermore, there were consistently more ears with SOAEs - and more SOAEs - in female ears compared with male ears. Thus it was also essential to ensure equal proportions of both genders in groups which were to be compared.

3.7.3. EFFECT OF AGE

For analysis of SOAE occurrence, frequency and amplitude, control subjects were split into two groups of approximately equal number (from 6-19 and 20-37 years old). There were approximately equal proportions of both left and right ears in juvenile and adult control subjects (juveniles = 28 of each side; adults = 37 left and 38 right).

For SOAE occurrence, there were no significant differences in the number of ears exhibiting SOAEs between juvenile and adult groups of control subjects (χ^2, $p > 0.10$). For female ears, juveniles had one or more SOAEs in 6 out of 24 ears, exhibiting a total of 8 SOAEs; whereas adults had one or more SOAEs in 14 out of 38 ears ($\chi^2 = 0.94$, $df = 1$, $p = 0.331$), exhibiting a total of 24 SOAEs. Similarly for male ears, juveniles had one or more SOAEs in 5 out of 32 ears, exhibiting a total of 12 SOAEs; whereas adults had one or more SOAEs in 2 out of 37 ears ($\chi^2 = 1.97$, $df = 1$, $p = 0.161$), exhibiting a total of 3 SOAEs.

For SOAE frequency, Figure 3.7.3.1. shows a scatter plot and regression analyses for the effect of age on control subjects (mean+/-SD: left ears = 2.04+/-0.88 kHz, n = 24, $p > 0.10$; right ears = 1.91+/-0.83 kHz, n = 23, $p > 0.10$). Similarly for SOAE amplitude, Figure 3.7.3.2. shows a scatter plot and regression analyses for the effect of age on control subjects (mean+/-SD: left ears = 4.08+/-3.57 dBSPL, n = 24, $p > 0.10$; right ears = 8.96+/-6.28 dBSPL, n = 23, $p > 0.10$).

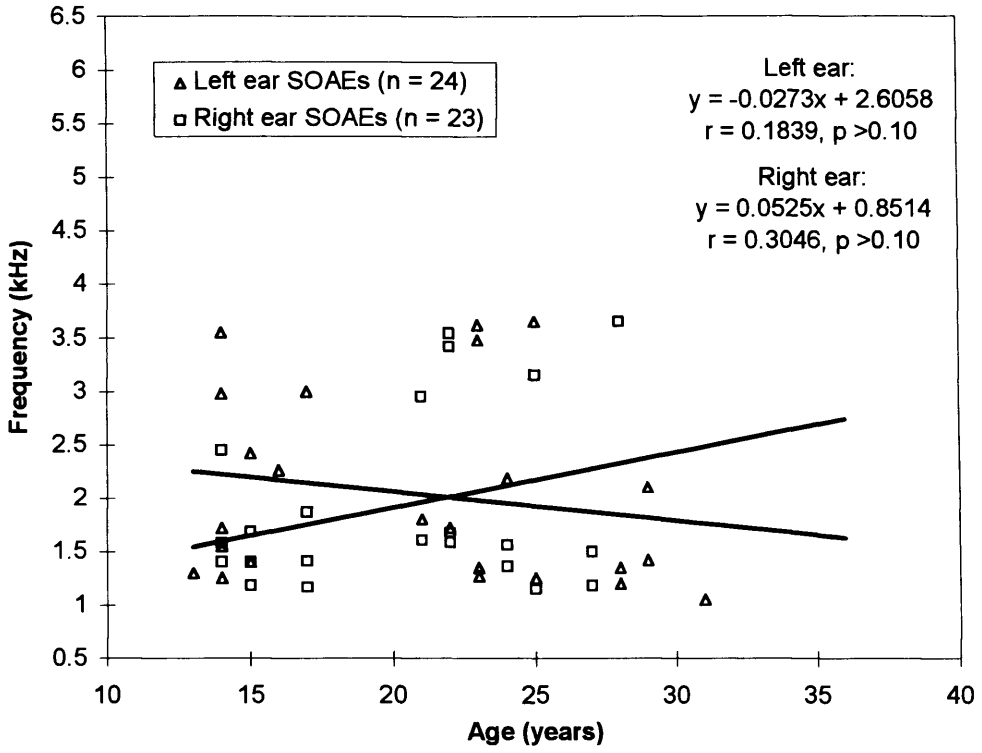


Fig. 3.7.3.1. SOAE frequency: Effect of age in controls

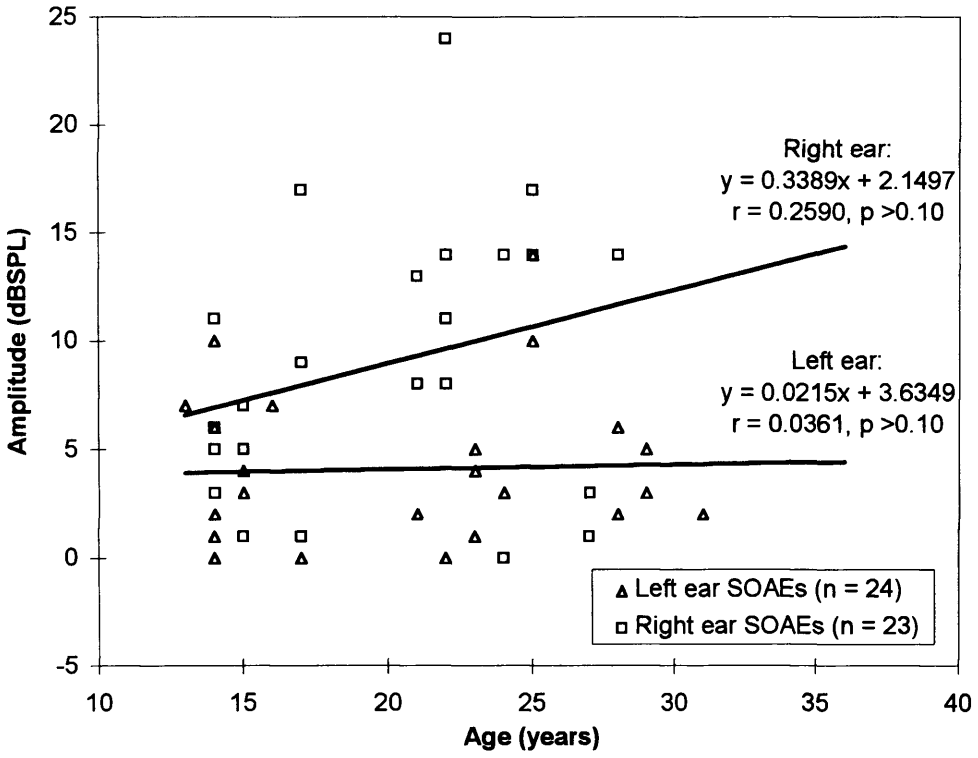


Fig. 3.7.3.2. SOAE amplitude: Effect of age in controls

For SOAE occurrence (number), there were no significant differences ($p > 0.10$) between juvenile and adult groups. Furthermore for both SOAE frequency (Hz) and amplitude (dBSPL), there was no correlation ($p > 0.10$) with age. Hence, there was no need to partition groups according to age.

3.7.4. EFFECT OF DOSE

For analysis of SOAE occurrence, frequency and amplitude, patients were split into two groups of approximately equal number (from 0-99 and 100-700 days total exposure). There were approximately equal proportions of both left and right ears in low and high dose patient groups (low dose patients = 17 of each side; high dose patients = 19 left and 20 right).

For SOAE occurrence, there were no significant differences in the number of ears exhibiting SOAEs between low and high dose patient groups (χ^2, $p > 0.10$). For female ears, low dose patients had one or more SOAEs in 6 out of 25 ears, exhibiting a total of 6 SOAEs; whereas high dose patients had one or more SOAEs in 3 out of 12 ears ($\chi^2 = 0.01$, $df = 1$, $p = 0.947$), exhibiting a total of 9 SOAEs. Similarly for male ears, low dose patients had one or more SOAEs in 3 out of 29 ears, exhibiting a total of 3 SOAEs; whereas high dose patients had no SOAEs in any of the 7 ears measured ($\chi^2 = 0.79$, $df = 1$, $p = 0.374$). None of the patients with bilateral sensorineural loss (an additional 2 female and 6 male ears, all in the high dose category), exhibited any SOAEs.

For SOAE frequency, Figure 3.7.4.1. shows a dose-response scatter plot for patients (mean+/-SD: left ears = 2.40+/-0.88 kHz, n = 6; right ears = 1.87+/-0.91 kHz, n = 12). Similarly for SOAE amplitude, Figure 3.7.4.2. shows a dose-response scatter plot for patients (mean+/-SD: left ears = 3.33+/-3.56 dBSPL, n = 6; right ears = 6.58+/-6.05 dBSPL, n = 12). It is apparent that SOAEs occur too infrequently in patients to assess a correlation between dose and either frequency (Hz) or amplitude (dBSPL).

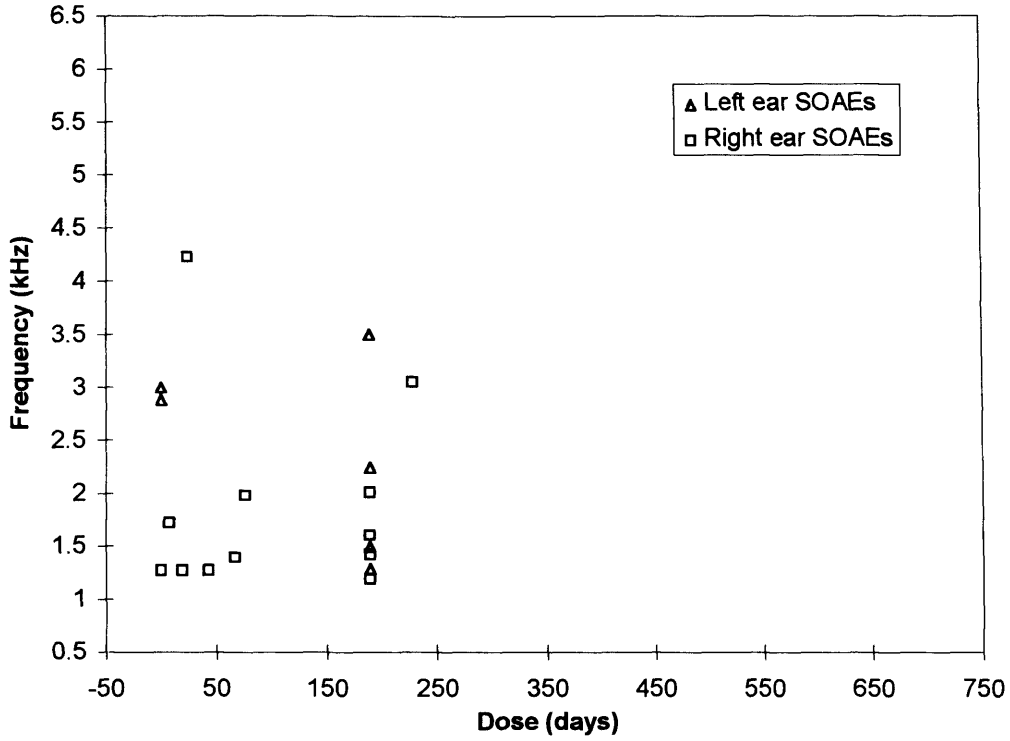


Fig. 3.7.4.1. SOAE frequency: Dose-response for patients passing PTA criteria

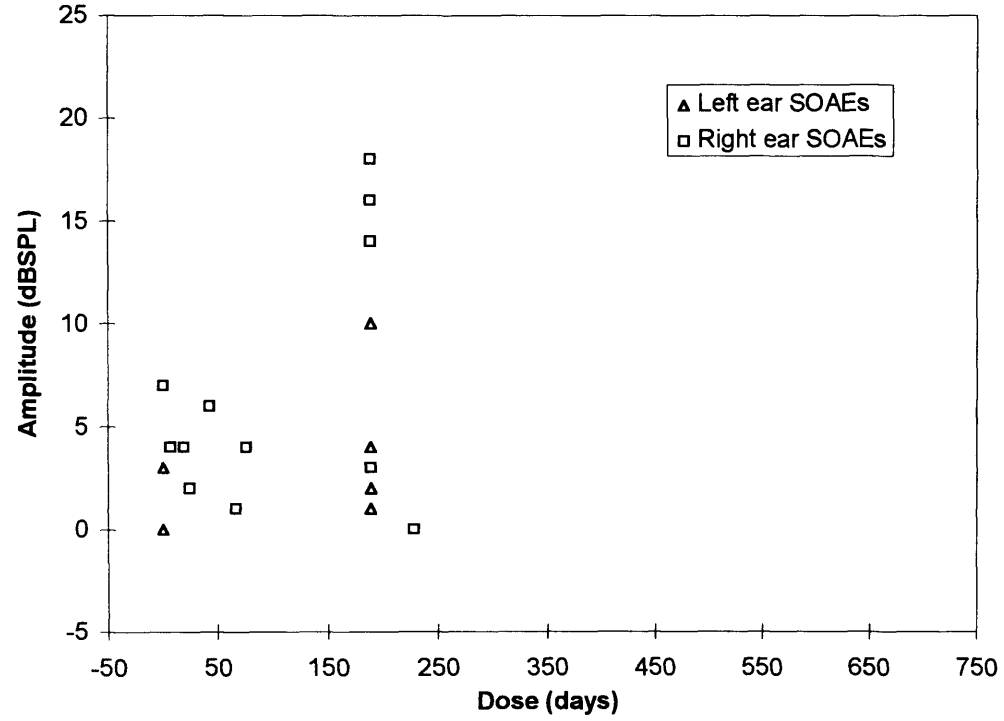


Fig. 3.7.4.2. SOAE amplitude: Dose-response for patients passing PTA criteria

3.7.5. CONTROLS VERSUS PATIENTS

There were approximately equal proportions of both genders in control and patient groups (for control subjects 29 out of 61 left ears and 30 out of 61 right ears were female; for patients 16 out of 32 left ears and 21 out of 39 right ears were female). Figure 3.7.5.1.A. shows the 6th order polynomial regression analyses of mean spectra for left ears (control subject n = 61, mean+/-SD averages = 205+/-63; patient n = 32, mean+/-SD averages = 183+/-75). Similarly, Figure 3.7.5.1.B. shows the 6th order polynomial regression analyses of mean spectra for right ears (control subject n = 61, mean+/-SD averages = 203+/-61; patient n = 39, mean+/-SD averages = 193+/-71).* Considering the frequencies over which SOAEs were designated (between 1001 and 6238 Hz), the mean +2SD was 3.1 dB (mean SD+/-SD: control left ears = 1.52+/-1.25 dB, and right ears = 1.51+/-1.33 dB; patient left ears = 1.57+/-1.3 dB and right ears = 1.53+/-1.4 dB; n = 430 frequency points). For both ears, control and patient lines converge between 2.5 and 3 kHz, whereas mean and mean+2SD lines converge between 4.5 and 5 kHz. Thus, the background 'noise' characteristics are: predictable (see regression equations); variable within the limits of SOAE designation (i.e. +3.1 dB is within +6 dB); and, very similar for control and patient groups (practically identical above 2.5-3 kHz).**

---

* With 511 frequency points per line, 48 ears per point, and 196 averages per ear; each line represents a summary of approximately 4.8 million numbers.

** It should be remembered that calculation of a mean noise spectrum will intrinsically include SOAEs.

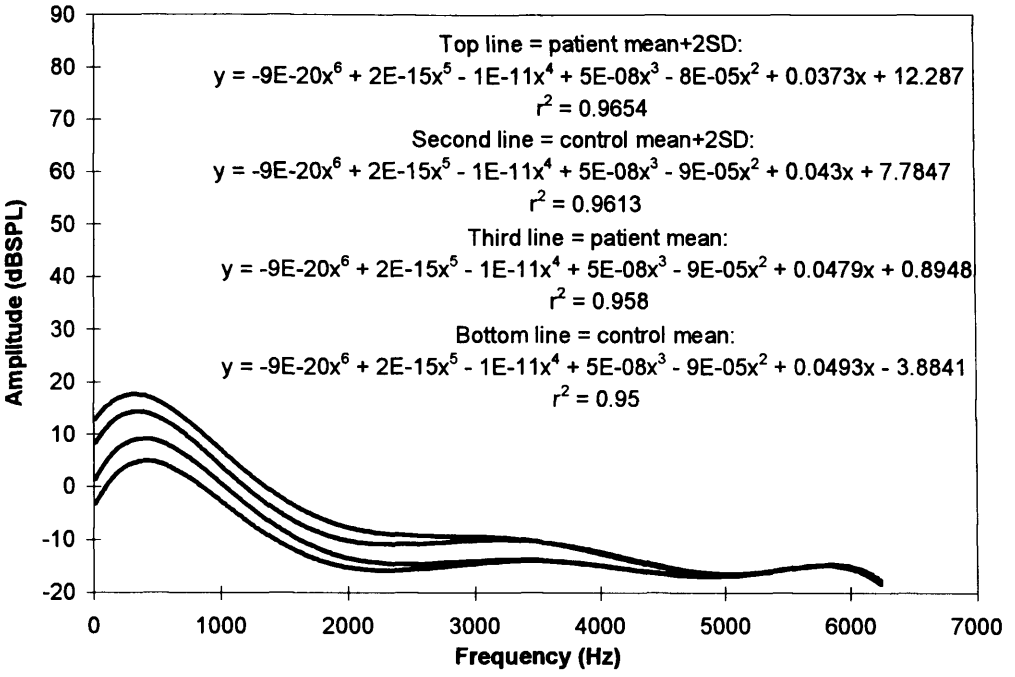


Fig. 3.7.5.1.A. Polynomial regression (6th order) of mean SOAE spectra for control and patient left ears

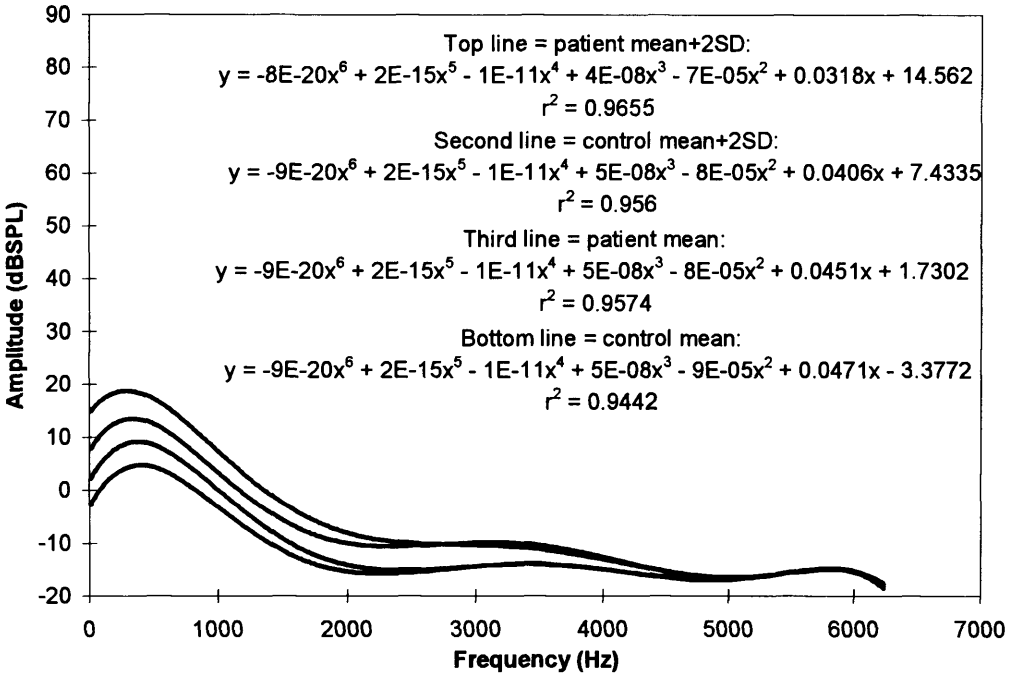


Fig. 3.7.5.1.B. Polynomial regression (6th order) of mean SOAE spectra for control and patient right ears

Figure 3.7.5.2. shows a scatter plot of SOAE amplitude versus frequency, summarising the relationship between control subjects (n = 24 left and 23 right ear SOAEs) and patients (n = 4 left and 11 right ear SOAEs; excluding those who had received no aminoglycoside exposure). Again there were approximately equal proportions of both left and right ears in control and patient groups (control subjects = 65 left and 66 right, patients = 31 left and 34 right).

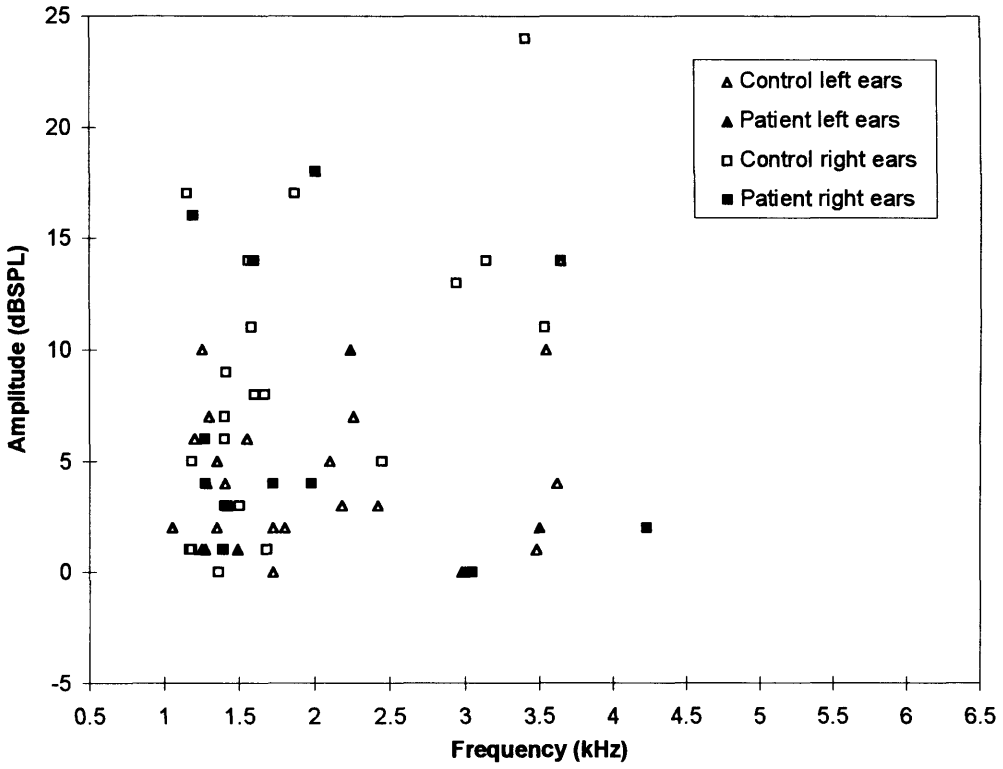


Fig. 3.7.5.2. Distribution of SOAEs in controls and patients

For SOAE occurrence, when excluding patients who had received no aminoglycoside exposure, there were no significant differences when comparing patient and control groups (χ^2, $p > 0.10$). For female ears, control subjects had one or more SOAEs in 20 out of 42 ears, exhibiting a total of 32 SOAEs; whereas patients had one or more SOAEs in 8 out of 28 ears ($\chi^2 = 2.54$, df = 1, $p = 0.111$), exhibiting a total of 14 SOAEs. Similarly for male ears, control subjects had one or more SOAEs in 7 out of 62 ears, exhibiting a total of 15 SOAEs; whereas patients had one or more SOAEs in 1 out of 28 ears ($\chi^2 = 1.42$, df = 1, $p = 0.234$), exhibiting a total of 1 SOAE.

For SOAE frequency (Hz), when excluding patients who had received no aminoglycoside exposure, there were no significant differences (all Type II T tests, $p = 0.852$ for left and 0.965 for right ears) between patients (mean+/-SD: left ears = 2.13+/-1.00 kHz, n = 4; right ears = 1.92+/-0.93 kHz, n = 11) and control subjects (mean+/-SD: left ears = 2.04+/-0.88 kHz, n = 24; right ears = 1.91+/-0.83 kHz, n = 23). Similarly for SOAE amplitude (dBSPL), when excluding patients who had received no aminoglycoside exposure, there were no significant differences (all Type II T tests, $p = 0.933$ for left and 0.305 for right ears) between patients (mean+/-SD: left ears = 4.25+/-4.03 dBSPL, n = 4; right ears = 6.55+/-6.35 dBSPL, n = 11) and control subjects (mean+/-SD: left ears = 4.08+/-3.57 dBSPL, n = 24; right ears = 8.96+/-6.29 dBSPL, n = 23).

Retrospective power analysis for frequency (Hz) of SOAEs reveals that with a mean standard deviation of 0.86 kHz (pooling control left and right ears), for 80% power and $p = 0.05$: to detect a difference of 1 kHz between means, each group needed to include 12 measures; and to detect a difference of 2 kHz between means, each group needed to include 3 measures. The actual number of patient measures (4 left, and 11 right ears) and control measures (24 left and 23 right ears) can therefore only be expected to have detected a significant difference of between 1-2 kHz (at $p = 0.05$) in 80 % of cases. Similarly, power

analysis for amplitude (dBSPL) of SOAEs reveals that with a mean standard deviation of 4.93 dB: to detect a difference of 6 dB between means, each group needed to include 11 measures; and to detect a difference of 9 dB between means, each group needed to include 5 measures. The actual number of measures (which were the same as for frequency above) can therefore only be expected to have detected a significant difference of between 6-9 dB (at $p = 0.05$) in 80 % of cases.

There were no significant differences ($p > 0.10$) in SOAE occurrence, frequency nor amplitude when comparing control and patient groups. However, the low occurrence of SOAEs - especially in patients - provides an extremely low level of statistical power for the detection of any differences in SOAEs.

3.8. OTO-ACOUSTIC EMISSIONS - CSSOAES

Only peaks at or above both 1 kHz* and -30 dBSPL were included as CSSOAEs. CSSOAE background noise was always from -40 up to and never exceeding -30 dBSPL, except where more variable below 1 kHz.** For an example of CSSOAE recording see Figure 3.9.4.1. later, where a graphical comparison with SOAEs facilitates interpretation.

3.8.1. TEST RE-TEST VARIABILITY

As for SOAEs, CSSOAE peaks repeated within +/-12.5 Hz were assumed to be variation of the same CSSOAE. For CSSOAE amplitude (dBSPL), repeated measurements in one representative control individual were found to be approximately equally stable, irrespective of the time of testing (see Table 3.8.1.). With increasing interval between measurements the amplitude variance increases for all assessed CSSOAEs regardless of absolute amplitude or frequency. Overall, the absolute amplitude measurements of the same CSSOAE appear stable irrespective of the frequency or ear. However, for all minute-minute measurements of the 3.149 kHz CSSOAE in the right ear the absolute amplitude was consistently lower than either AM-PM or week-week measurements. Thus as for SOAEs, minute-minute measurements of CSSOAE amplitude appear more stable - although not necessarily representative - suggesting that AM-PM measurements would be a better indicator of long term reproducibility.

---

* It should be noted that all CSSOAE frequency values presented here are too high by 12.5 Hz due to an error in the ILO88 software (Smurzynski & Probst, 1996).

** In addition, below 1 kHz CSSOAEs appear to be either 'drawn up' or 'swamped' by low frequency noise.

Ear	Frequency (kHz)	Minute-Minute Mean+/-SD (dBSPL)	AM-PM Mean+/-SD (dBSPL)	Week-Week Mean+/-SD (dBSPL)
Left	1.245	-3.08+/-0.22	-1.56+/-0.59	-2.32+/-0.75
	3.662	-0.22+/-0.22	-0.87+/-0.68	-0.97+/-0.70
Right	1.135	4.38+/-0.15	2.15+/-0.69	2.33+/-0.83
	3.149	-11.20+/-0.76	-1.62+/-0.61	-1.08+/-2.72

TABLE. 3.8.1. Test-retest variability of CSSOAE amplitude (dBSPL): Minute-minute over ten minutes (n = 10 sequential runs, after 150+/-2 averages), AM-PM over one week (n = 5 days AM and PM, after 260+/-2 averages) and week-week over one year (n = 47 weeks, after 150+/-2 averages).

3.8.2. GENDER AND AURAL ASYMMETRY

For analysis of CSSOAE occurrence, frequency and amplitude, control subjects were separated according to sex (female or male) and ear (left or right).

Figure 3.8.2. shows a scatter plot of amplitude versus frequency, summarising the relationship between females (n = 34 left ears, and 35 right ears) and males (n = 36 left ears, and 34 right ears). There were significantly more left ears with CSSOAEs than without CSSOAEs in females when compared with males (25 female and 17 male left ears had peaks; χ^2 = 5.04, df = 1, p = 0.025). There were no other significant findings (χ^2, p >0.05) concerning the number of ears with CSSOAEs (although 24 female and 16 male right ears had peaks; χ^2 = 3.27, df = 1, p = 0.070). Overall, there were one or more CSSOAEs in: 74 % of female left ears; 69 % of female right ears; and, 47 % of both male left and right ears. The maximum number of CSSOAEs was similar for all groups: female left ears = 8; female right ears = 6; male left ears = 5; and, male right ears = 8 peaks per ear. There were more peaks in female than male ears, and more peaks in right ears than left ears

(CSSOAEs in: female left ears = 69; female right ears = 83; male left ears = 30; and, male right ears = 45). Overall, there were twice as many female than male CSSOAEs, and approximately one-third more CSSOAEs in right ears than left ears. As there was evidently a greater effect of gender on CSSOAEs, further analyses of CSSOAE occurrence were separated into female and male groups.

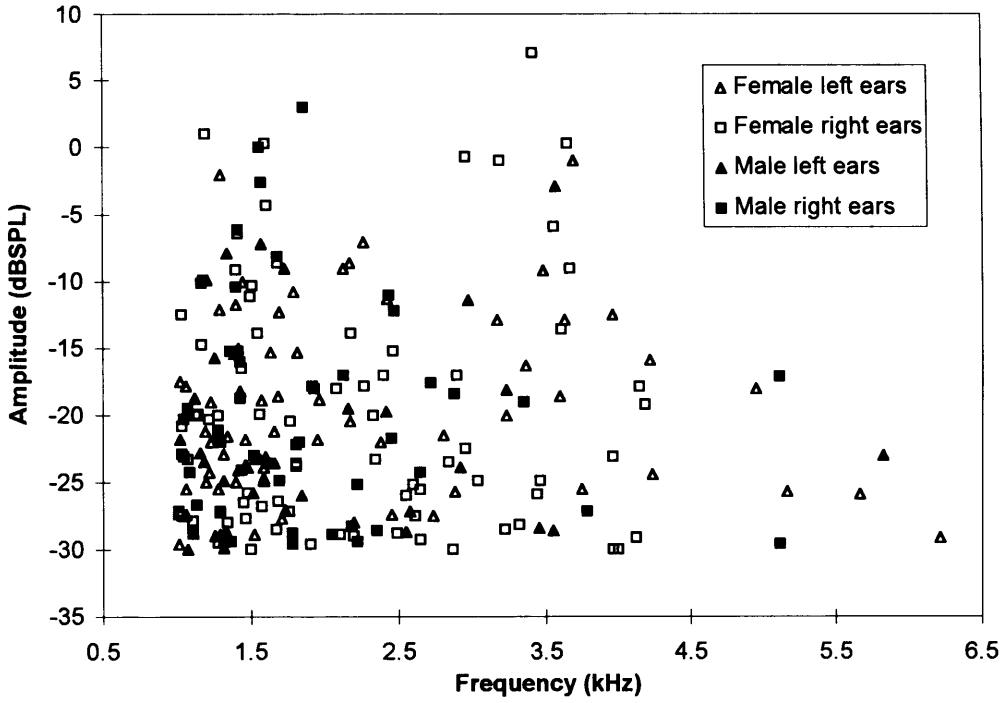


Fig. 3.8.2. Distribution of CSSOAEs in male and female controls

The frequency of female left ear CSSOAEs (mean+/-SD = 2.19+/-1.21 kHz), female right ear CSSOAEs (2.13+/-0.95 kHz), male left ear CSSOAEs (2+/-1.09 kHz), and male right ear CSSOAEs (1.95+/-0.93 kHz) were not significantly different (all Mann-Whitney tests, $p > 0.10$). The maximum CSSOAE frequency was similar for all groups: female left ears = 6.21 kHz; female right ears = 4.19 kHz; male left ears = 5.82 kHz; and, male right ears = 5.12 kHz.

The maximum CSSOAE amplitude was similar for all groups: female left ears = -1.0 dBSPL; female right ears = 7.1 dBSPL; male left ears = -2.9 dBSPL; and, male right ears = 3.0 dBSPL. There were no significant findings ($p > 0.10$, all Mann-Whitney tests, except for Type II comparison of left and right in males) concerning the amplitude of CSSOAEs (mean+/-SD: female left ear = -20.0+/-7.01 dBSPL; female right ear = -20.1+/-8.96 dBSPL; male left ears = -21.1+/-7.47 dBSPL; and, male right ears = -19.9+/-8.18 dBSPL).

For CSSOAE frequency (Hz) and amplitude (dBSPL), there were no significant differences ($p > 0.10$) with gender or ear. Considering CSSOAE occurrence, there were consistently more CSSOAEs in right ears compared with left ears (but not more right ears with CSSOAEs). Therefore, there must have been more right than left ear multiple emitters. Thus it was essential to ensure equal proportions of both ears in groups which were to be compared. Furthermore, there were consistently more ears with CSSOAEs - and more CSSOAEs - in female ears compared with male ears. Thus it was also essential to ensure equal proportions of both genders in groups which were to be compared.

3.8.3. EFFECT OF AGE

For analysis of CSSOAE occurrence, frequency and amplitude, control subjects were split into two groups of approximately equal number (from 6-19 and 20-37 years old). There were approximately equal proportions of both left and right ears in juvenile and adult control subjects (juveniles = 30 of each side; adults = 40 left and 39 right).

For CSSOAE occurrence, females had a significantly higher proportion of CSSOAEs in adult ears, whereas males had a significantly higher proportion of CSSOAEs in juvenile ears. For female ears, juveniles had one or more CSSOAEs in 15 out of 28 ears, exhibiting a total of 37 CSSOAEs; whereas adults had one or more CSSOAEs in 34 out of 41 ears (χ^2 = 6.97, df = 1, p = 0.008), exhibiting a total of 115 CSSOAEs. Similarly for male ears, juveniles had one or more CSSOAEs in 21 out of 32 ears, exhibiting a total of 48 CSSOAEs; whereas adults had one or more CSSOAEs in 12 out of 38 ears (χ^2 = 8.08, df = 1, p = 0.005), exhibiting a total of 27 CSSOAEs. The implication is that with increasing age females gain and males loose CSSOAEs.

For CSSOAE frequency, Figure 3.8.3.1. shows a scatter plot and regression analyses for the effect of age on control subjects (mean+/-SD: left ears = 2.13+/-1.17 kHz, n = 99, p >0.10; right ears = 2.07+/-0.94 kHz, n = 128, p >0.10). Similarly for CSSOAE amplitude, Figure 3.8.3.2. shows a scatter plot and regression analyses for the effect of age on control subjects (mean+/-SD: left ears = -20.30+/-7.13 dBSPL, n = 99, p >0.10; right ears = -20.02+/-8.67 dBSPL, n = 128, p >0.10).

For CSSOAE occurrence (number), there were gender-specific significant differences (p <0.01) between juvenile and adult groups which were not as great as the effect of gender alone (see 3.8.2.). Furthermore for both CSSOAE frequency (Hz) and amplitude (dBSPL), there was no correlation (p >0.10) with age. Hence, there was no need to partition groups according to age.

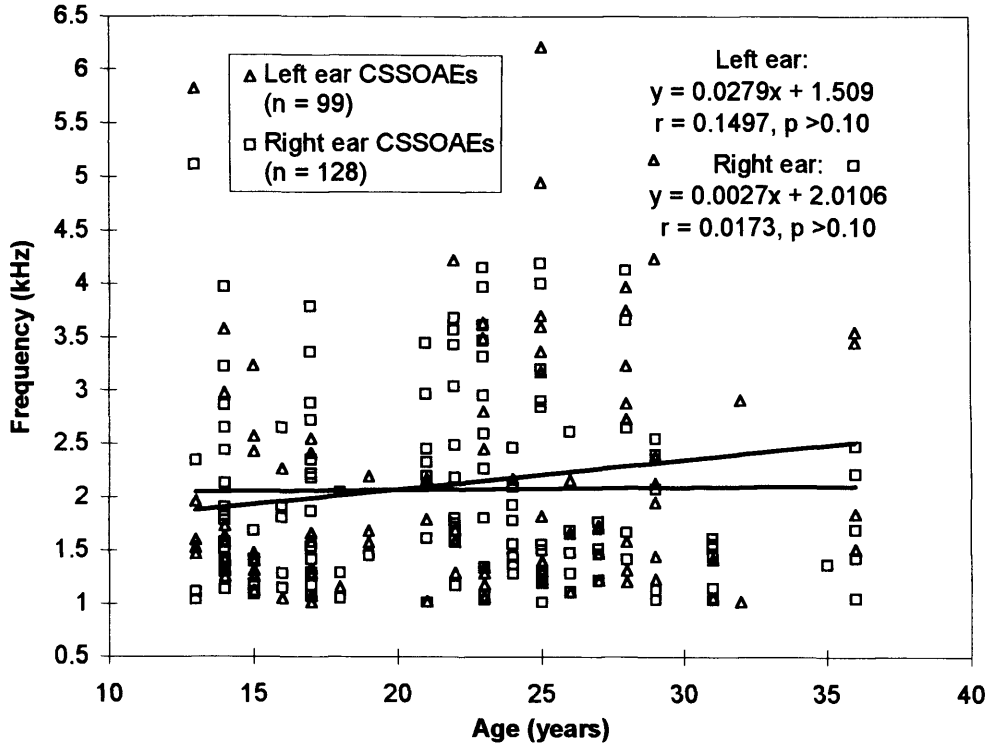


Fig. 3.8.3.1. CSSOAE frequency: Effect of age in controls

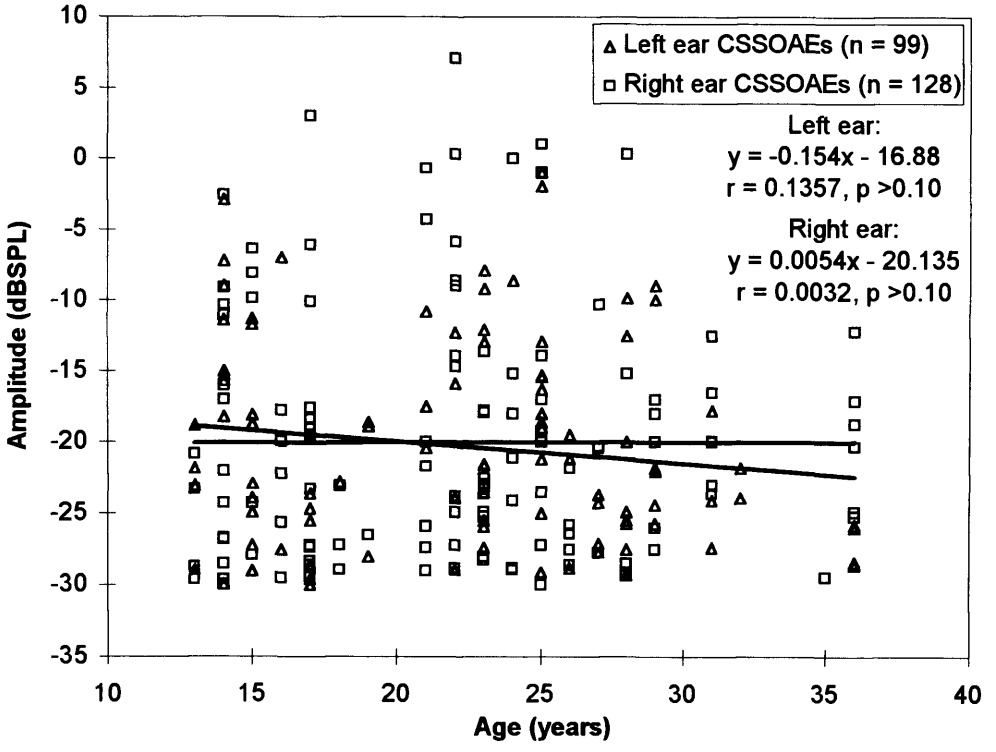


Fig. 3.8.3.2. CSSOAE amplitude: Effect of age in controls

3.8.4. EFFECT OF DOSE

For analysis of CSSOAE occurrence, frequency and amplitude, patients were split into two groups of approximately equal number (from 0-99 and 100-700 days total exposure). There were approximately equal proportions of both left and right ears in low and high dose patient groups (low dose patients = 24 left and 25 right; high dose patients = 27 left and 28 right).

For CSSOAE occurrence, there were no significant differences in the number of ears exhibiting CSSOAEs between low and high dose patient groups (all χ^2, $p > 0.05$). For female ears, low dose patients had one or more CSSOAEs in 17 out of 25 ears, exhibiting a total of 71 CSSOAEs; whereas high dose patients had one or more CSSOAEs in 18 out of 29 ears, exhibiting a total of 78 CSSOAEs. Similarly for male ears, low dose patients had one or more CSSOAEs in 14 out of 24 ears, exhibiting a total of 43 CSSOAEs; whereas high dose patients had one or more CSSOAEs in 10 out of 26 ears, exhibiting a total of 14 CSSOAEs. Incidentally, of the patients with bilateral sensorineural loss (all in the high dose category) there were 2 male ears and 1 female ear each with 1 CSSOAE, and 1 female ear with 2 CSSOAEs (out of 2 female and 8 male ears in total).

Neither CSSOAE frequency (Hz) nor amplitude (dBSPL) were found to correlate with dose in patients ($p > 0.10$). Dose-response scatter plots and regression analyses are shown separately, for frequency and amplitude in Figures 3.8.4.1. and 3.8.4.2. respectively (in both cases Figure A. shows left ears and B. shows right ears).

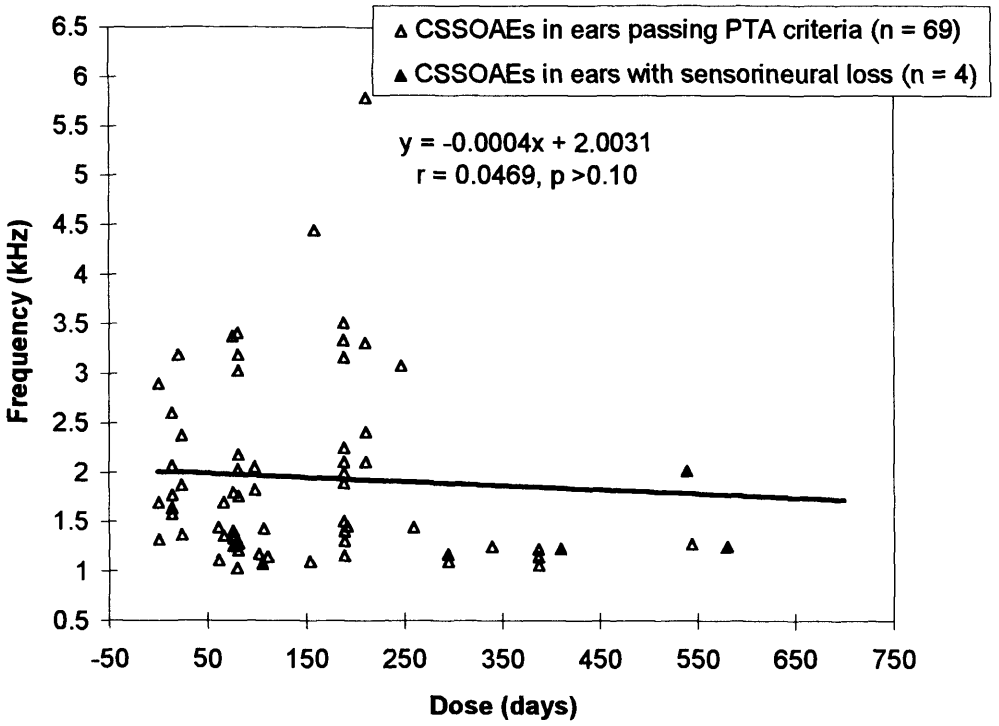


Fig. 3.8.4.1.A. CSSOAE frequency: Dose-response for left ears (n = 51). Mean frequency+/-SD = 2.04+/-0.94 kHz (n = 69).

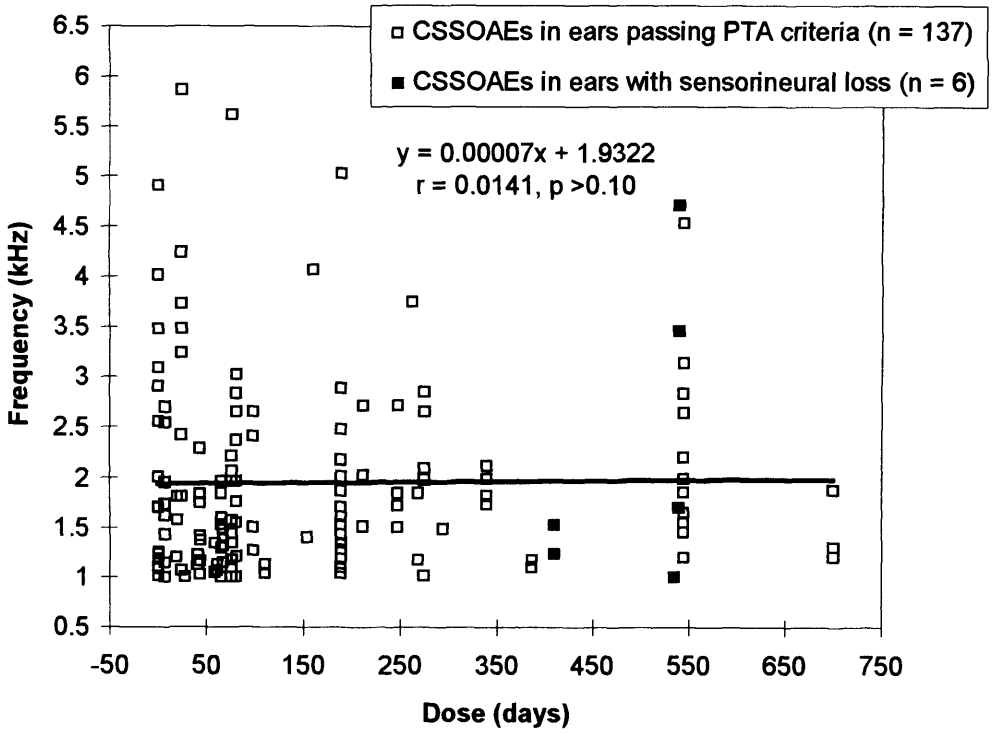


Fig. 3.8.4.1.B. CSSOAE frequency: Dose-response for right ears (n = 53). Mean frequency+/-SD = 1.94+/-0.97 kHz (n = 137).

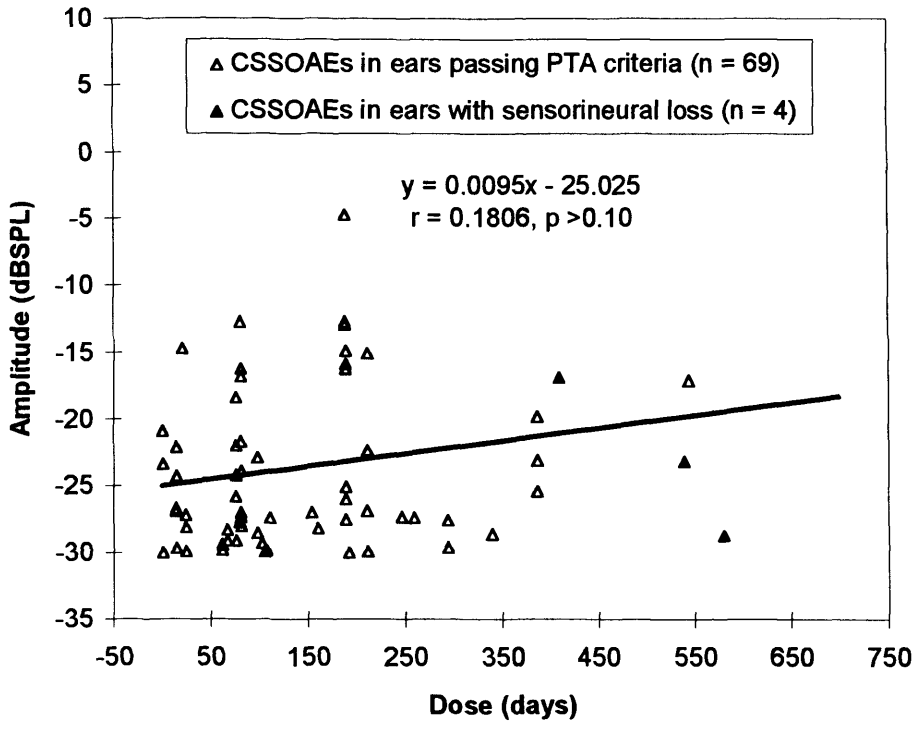


Fig. 3.8.4.2.A. CSSOAE amplitude: Dose-response for left ears (n = 51). Mean amplitude+/-SD = -22.90+/-6.50 dBSPL (69).

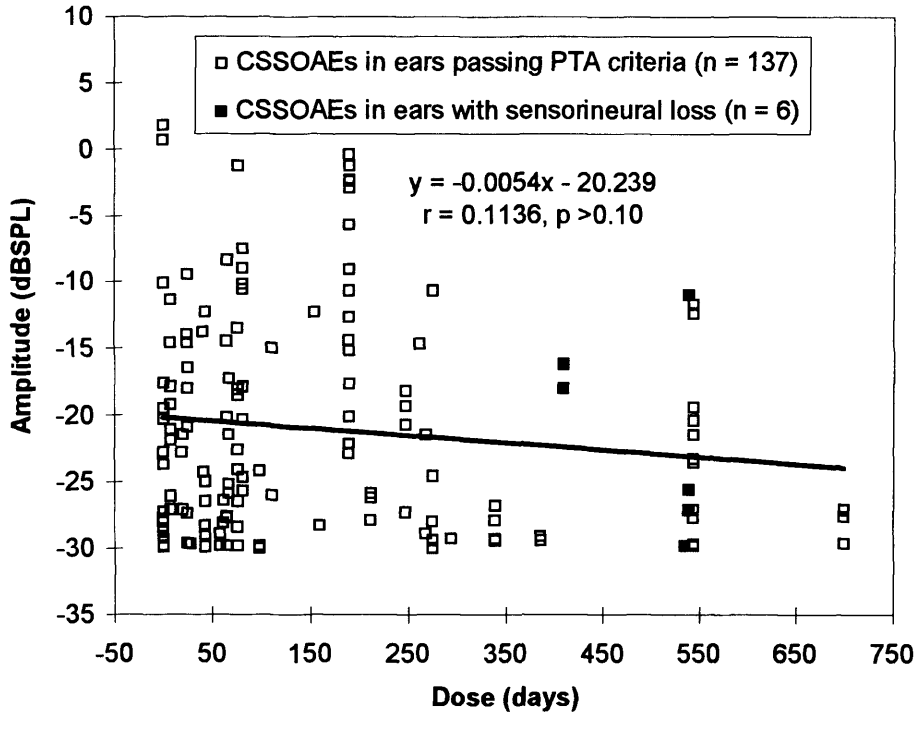


Fig. 3.8.4.2.B. CSSOAE amplitude: Dose-response for right ears (n = 53). Mean amplitude+/-SD = -21.10+/-8.30 dBSPL (n = 137).

3.8.5. CONTROLS VERSUS PATIENTS

Figure 3.8.5. shows a scatter plot of CSSOAE amplitude versus frequency, summarising the relationship between control subjects (n = 99 left and 128 right ear CSSOAEs) and patients (n = 68 left and 124 right ear CSSOAEs; excluding those who had received no aminoglycoside exposure, but including those with bilateral sensorineural loss). There were approximately equal proportions of both left and right ears in control and patient groups (control subjects = 70 left and 69 right, patients = 46 left and 47 right).

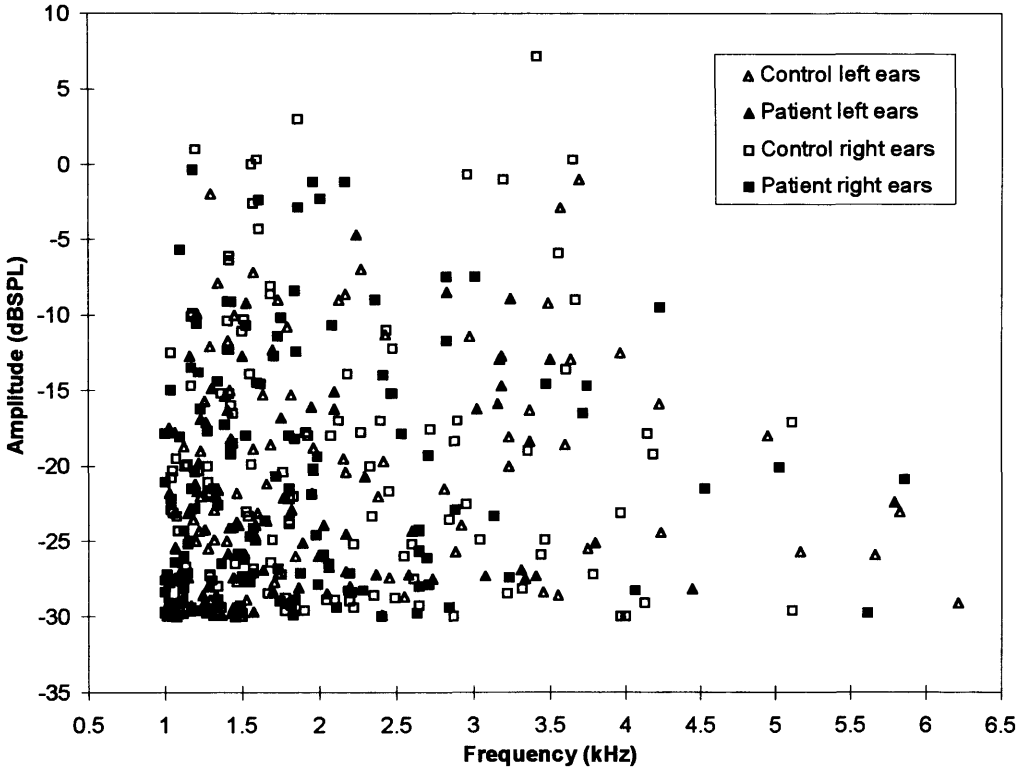


Fig. 3.8.5. Distribution of CSSOAEs in controls and patients

For CSSOAE occurrence, when excluding patients who had received no aminoglycoside exposure and excluding patients with bilateral sensorineural loss, there were no significant differences between control and patient groups (all χ^2, $p > 0.05$). For female ears, control subjects had one or more CSSOAEs in 49 out of 69 ears, exhibiting a

total of 152 CSSOAEs; whereas patients had one or more CSSOAEs in 31 out of 50 ears, exhibiting a total of 142 CSSOAEs. Similarly for male ears, control subjects had one or more CSSOAEs in 33 out of 70 ears, exhibiting a total of 75 CSSOAEs; whereas patients had one or more CSSOAEs in 20 out of 43 ears, exhibiting a total of 45 CSSOAEs. Similarly, when excluding patients who had received no aminoglycoside exposure but including patients with bilateral sensorineural loss, there were no significant differences between control and patient groups (all χ^2, $p > 0.05$). For female ears, patients had one or more CSSOAEs in 33 out of 52 ears, exhibiting a total of 145 CSSOAEs. Whereas, for male ears, patients had one or more CSSOAEs in 22 out of 29 ears, exhibiting a total of 47 CSSOAEs.

For CSSOAE frequency (Hz), when excluding patients who had received no aminoglycoside exposure and excluding patients with bilateral sensorineural loss, there were no significant differences ($p > 0.10$, both Mann-Whitney tests) between patients (mean+/-SD: left ears = 2.04+/-0.95 kHz, n = 66; right ears = 1.92+/-0.94 kHz, n = 121) and control subjects (mean+/-SD: left ears = 2.13+/-0.94 kHz, n = 99; right ears = 2.07+/-1.17 kHz, n = 128). Similarly, when excluding patients who had received no aminoglycoside exposure but including patients with bilateral sensorineural loss, there were no significant differences ($p > 0.10$, both Mann-Whitney tests) between patients (mean+/-SD: left ears = 2.01+/-0.95 kHz, n = 68; right ears = 1.90+/-0.93 kHz, n = 124) and control subjects.

For CSSOAE amplitude (dBSPL), when excluding patients who had received no aminoglycoside exposure and excluding patients with bilateral sensorineural loss, the only significant difference between patients (mean+/-SD: left ears = -22.85+/-6.58 dBSPL, n = 66; right ears = -21.04+/-8.03 dBSPL, n = 121) and control subjects (mean+/-SD: left ears = -20.32+/-7.13 dBSPL, n = 99; right ears = -22.02+/-8.67 dBSPL, n = 128), was for mean amplitude in left ears (Type II T test, p = 0.023). However, when excluding patients who had received

no aminoglycoside exposure but including patients with bilateral sensorineural loss, this single significant difference between patients (mean+/-SD: left ears = -22.87+/-6.58 dBSPL, n = 68; right ears = -21.05+/-7.99 dBSPL, n = 124) and control subjects disappeared (Type III T test, $p > 0.992$). There were no other significant differences (all Mann-Whitney tests, $p > 0.10$) in CSSOAE amplitude between patients and control subjects.

Retrospective power analysis for frequency (Hz) of CSSOAEs reveals that with a mean standard deviation of 1.06 kHz (pooling control left and right ears), for 80% power and $p = 0.05$: to detect a difference of 400 Hz between means, each group needed to include 109 measures; and to detect a difference of 500 Hz between means, each group needed to include 70 measures. The actual number of patient measures (66-68 left, and 121-124 right ear CSSOAEs; depending on hypothesis) and control measures (99 left and 128 right ear CSSOAEs) can therefore be expected to have detected a significant difference of 500 Hz (at $p = 0.05$) in 80 % of cases. Similarly, power analysis for amplitude (dBSPL) of CSSOAEs reveals that with a mean standard deviation of 7.90 dB: to detect a difference of 4 dB between means, each group needed to include 61 measures; and to detect a difference of 3 dB between means, each group needed to include 108 measures. The actual number of measures (which were the same as for frequency above) can therefore be expected to have detected a significant difference of 3 dB (at $p = 0.05$) in 80 % of cases.

Hence, for CSSOAEs, sample size was sufficient to detect a difference between patient and control groups of 500 Hz in frequency and 3 dB in amplitude. Inter-supporting significant differences in CSSOAE occurrence, frequency and amplitude were not apparent, even when including patients with bilateral sensorineural loss.

3.9. CROSS-TEST COMPARISONS

For control subjects only, it was also possible to correlate between different tests to establish the degree of any relationship.

3.9.1. DPOAES AND TYMPANOMETRY

Input-output function -10 dBSPL iso-criteria at 2, 4, 6 and 8 kHz were found not to correlate with tympanometry measures ($p > 0.05$). There were a few anomalous exceptions, a small proportion of the variability of iso-criteria at 4 and 6 kHz was significantly attributed to tympanometry pressure ($r = 0.28$ and 0.22, $p < 0.02$ and 0.05 respectively), although at both frequencies this was for the left ears only. In addition at 4 kHz there was also a correlation with tympanometry compliance ($r = 0.32$, $p < 0.01$), although again only for left ears. Thus for all frequencies tested, no correlation occurred that was consistent in both left and right ears. Scatter plots and regression analyses are shown, for all four frequencies: for the effect of compliance in Figures 3.9.1.1., 3.9.1.3., 3.9.1.5. and 3.9.1.7. respectively; and, for the effect of peak pressure in Figures 3.9.1.2., 3.9.1.4., 3.9.1.6. and 3.9.1.8. respectively. In all cases, ears previously excluded on grounds of poor tympanometry were included. However, it should be remembered that the majority of tympanometry measures were within the excepted normal clinical ranges (see methods 2.2.1.; results 3.1.4.).

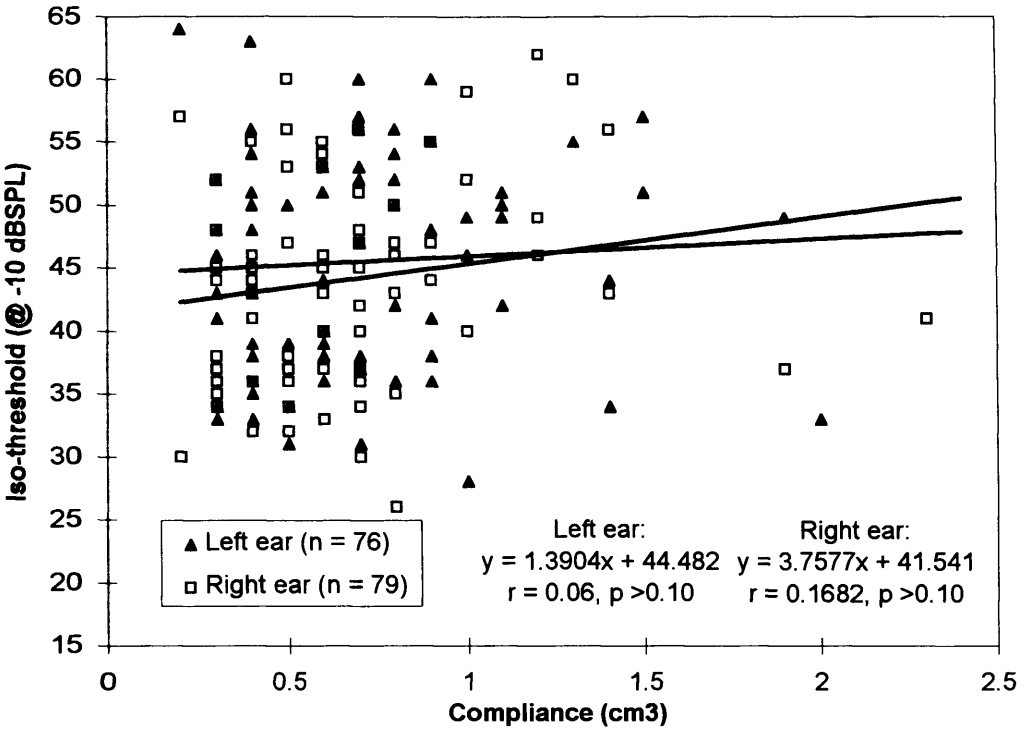


Fig. 3.9.1.1. 2 kHz DPOAE growth rate iso-thresholds: Effect of tympanometry compliance in controls

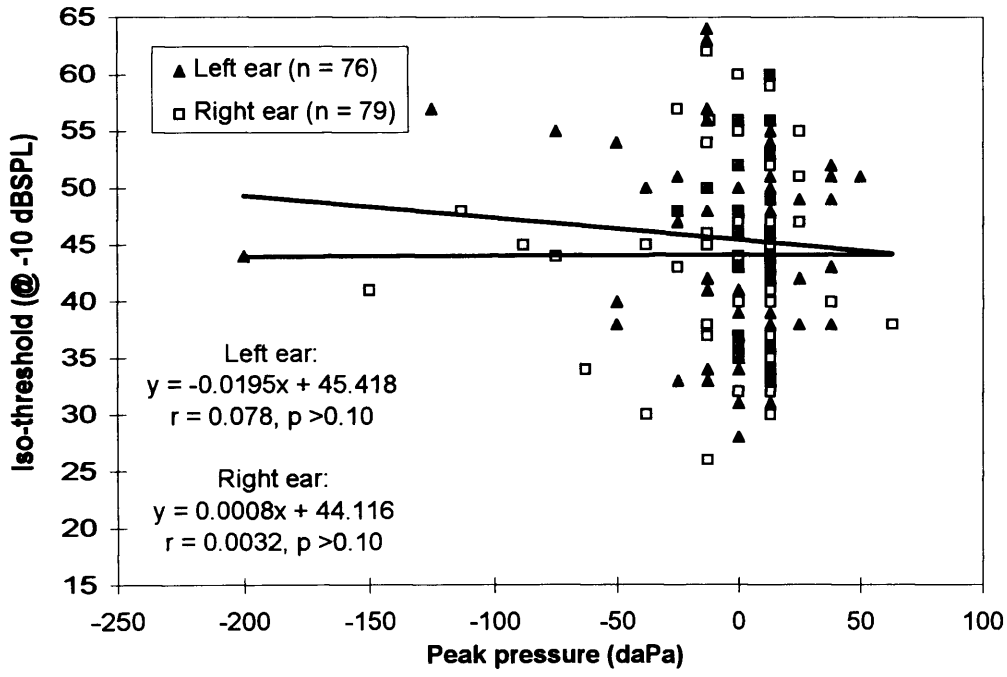


Fig. 3.9.1.2. 2 kHz DPOAE growth rate iso-thresholds: Effect of tympanometry pressure in controls

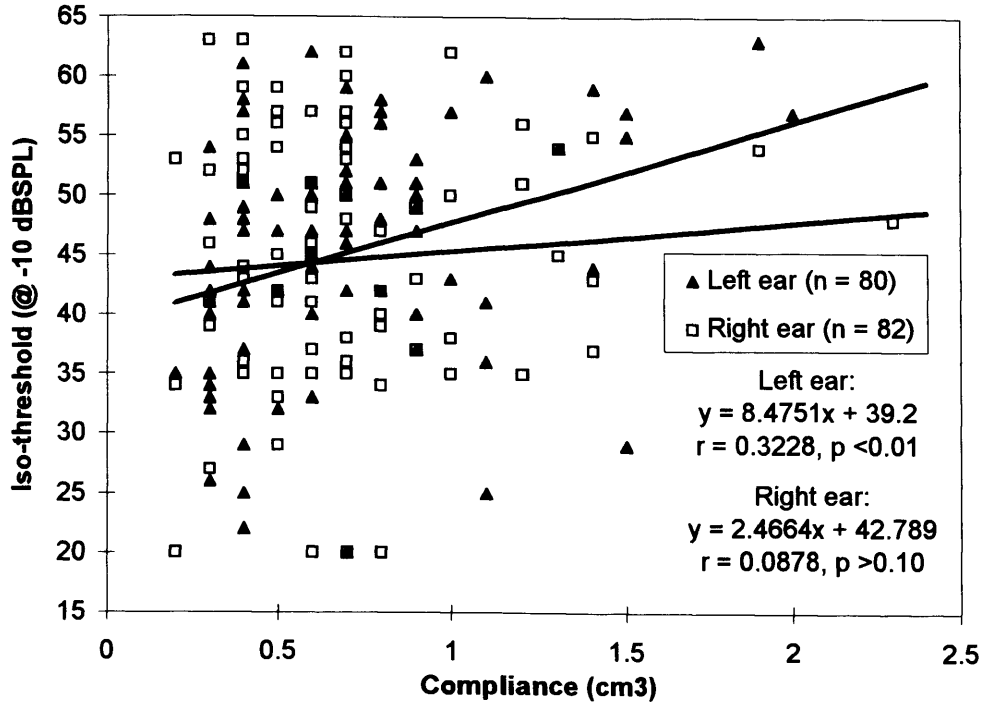


Fig. 3.9.1.3. 4 kHz DPOAE growth rate iso-thresholds: Effect of tympanometry compliance in controls

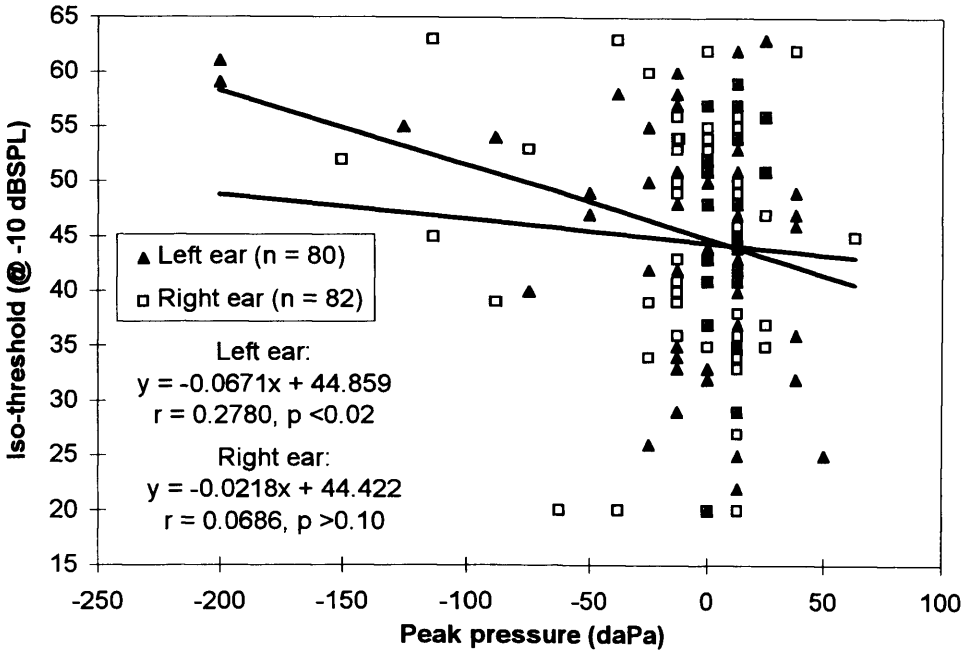


Fig. 3.9.1.4. 4 kHz DPOAE growth rate iso-thresholds: Effect of tympanometry pressure in controls

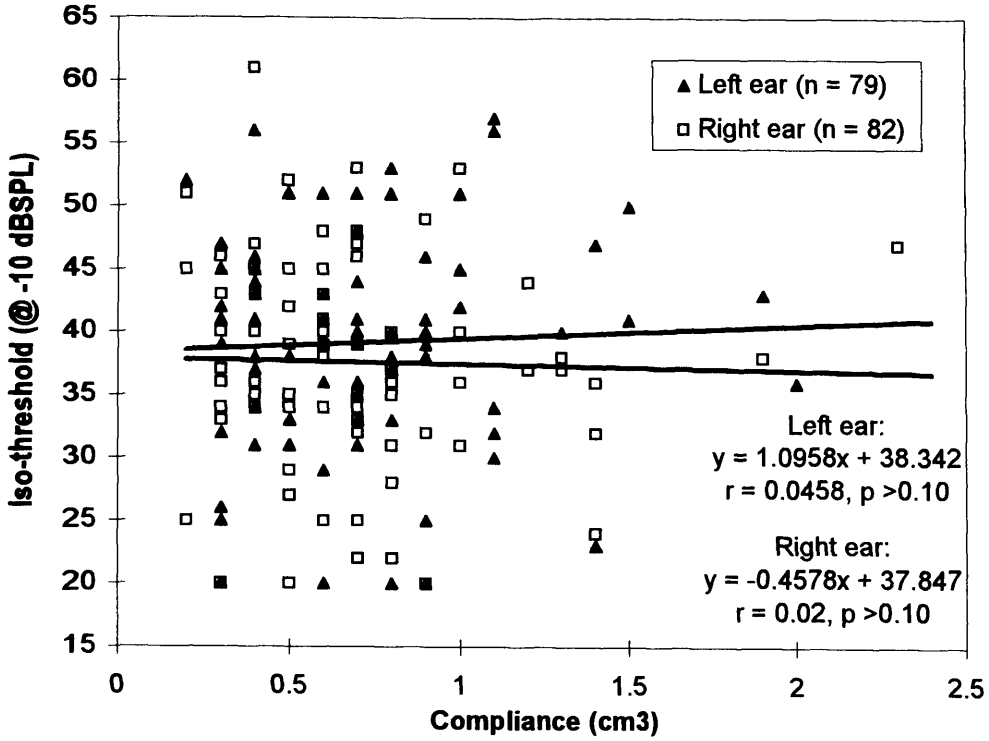


Fig. 3.9.1.5. 6 kHz growth rate iso-thresholds: Effect of tympanometry compliance in controls

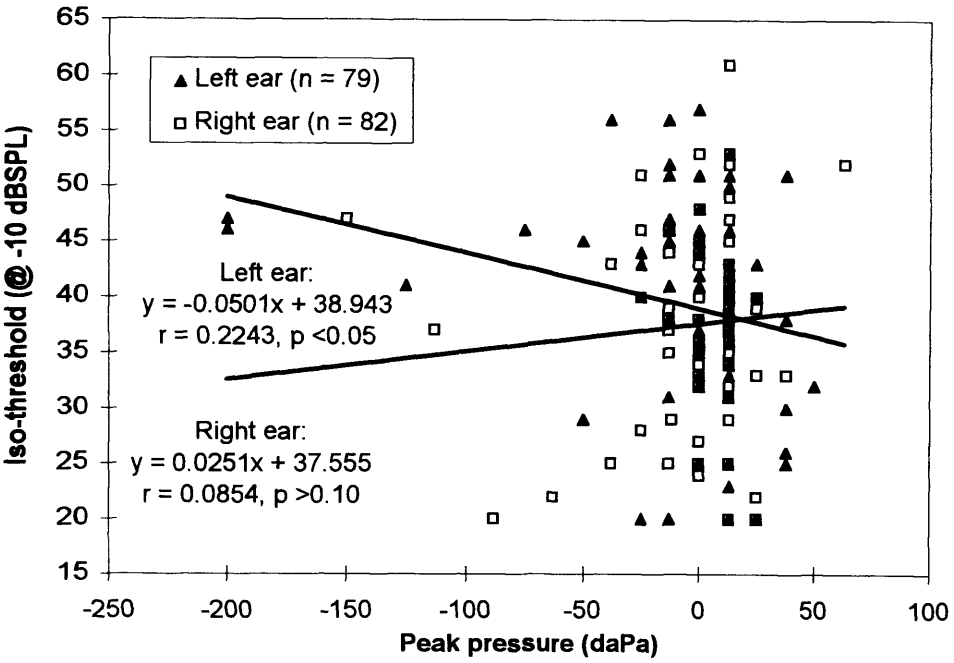


Fig. 3.9.1.6. 6 kHz DPOAE growth rate iso-thresholds: Effect of tympanometry pressure in controls

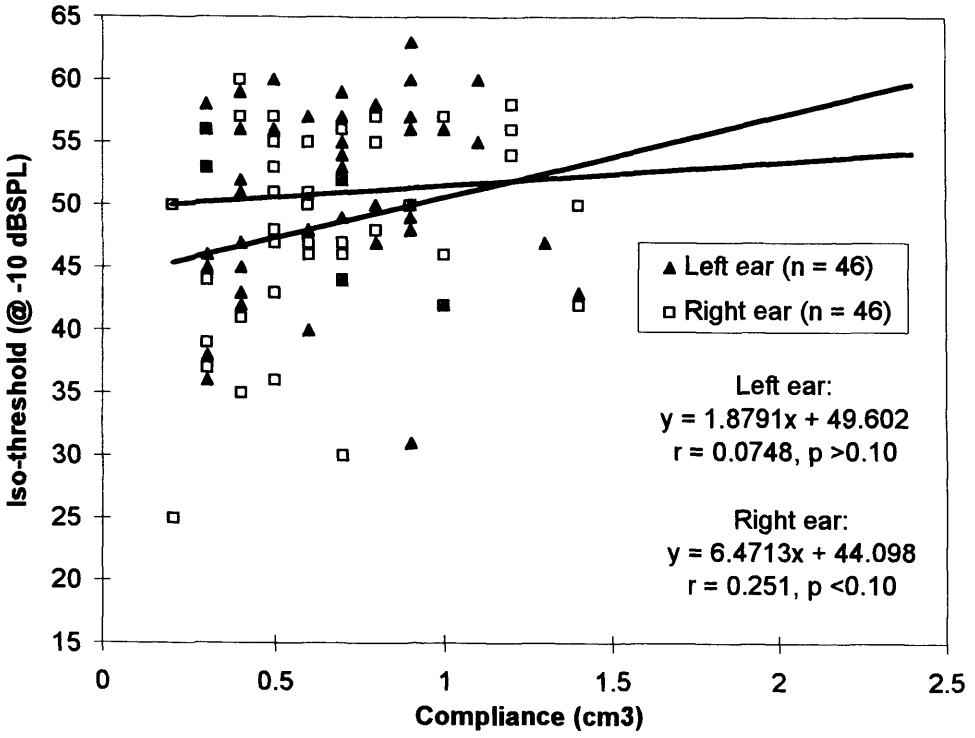


Fig. 3.9.1.7. 8 kHz growth rate iso-thresholds: Effect of tympanometry compliance in controls

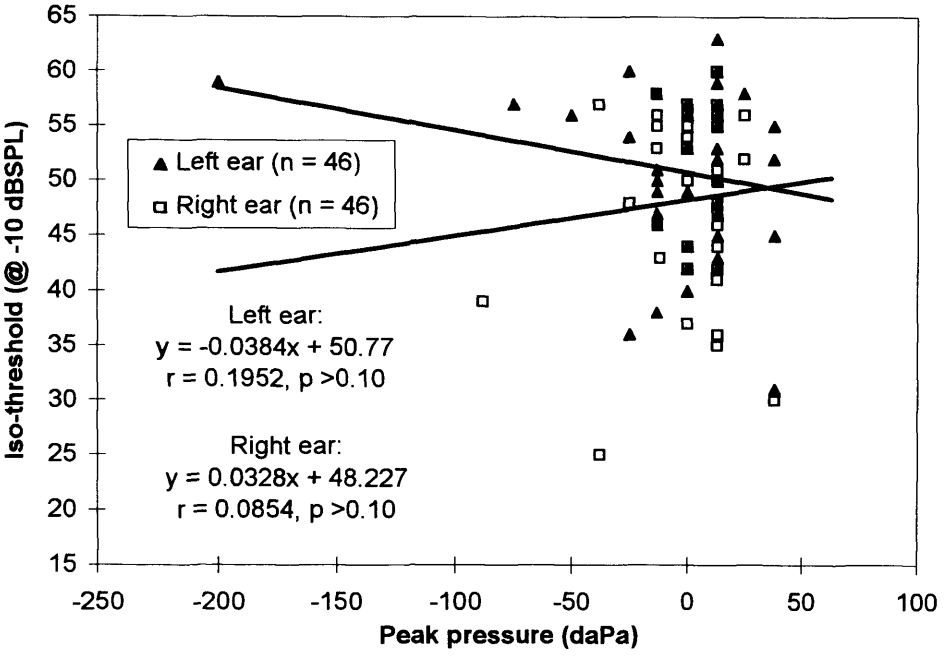


Fig. 3.9.1.8. 8 kHz DPOAE growth rate iso-thresholds: Effect of tympanometry pressure in controls

3.9.2. DPOAES AND FREQUENCY RESOLUTION

Input-output function -10 dBSPL iso-criteria at 2, 4 and 8 kHz were found not to correlate with the difference (dB) between thresholds at masking extremes ($p > 0.10$). Scatter plots and regression analyses are shown, for all three frequencies in Figures 3.9.2.1., 3.9.2.2. and 3.9.2.3. respectively.

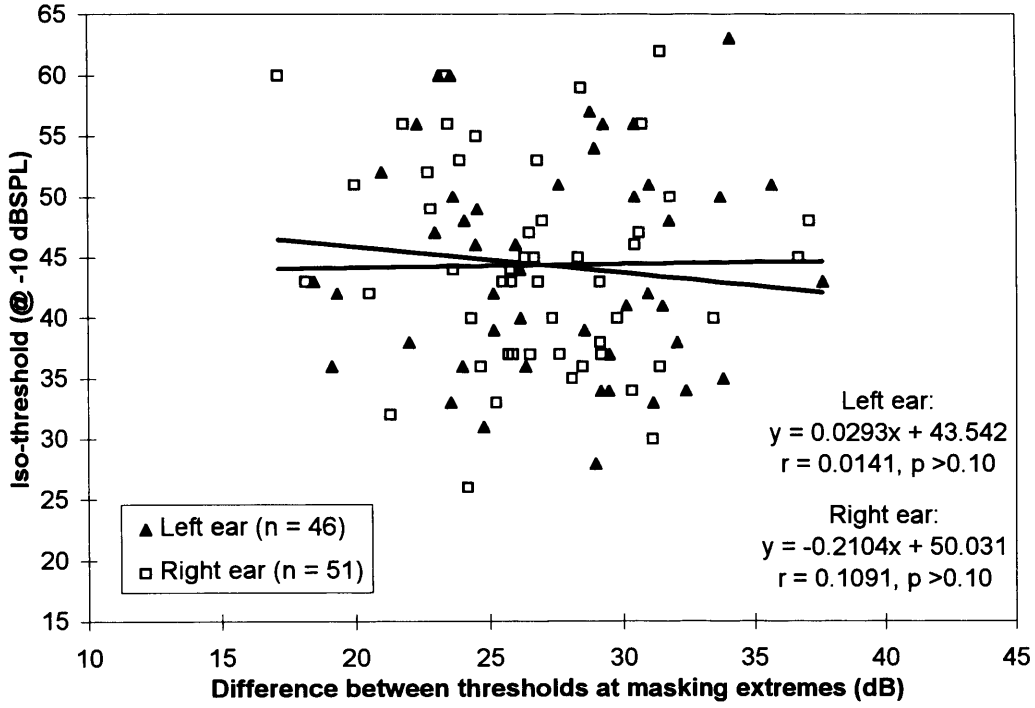


Fig. 3.9.2.1. Relationship between 2 kHz DPOAE growth rate iso-threshold and frequency resolution in controls

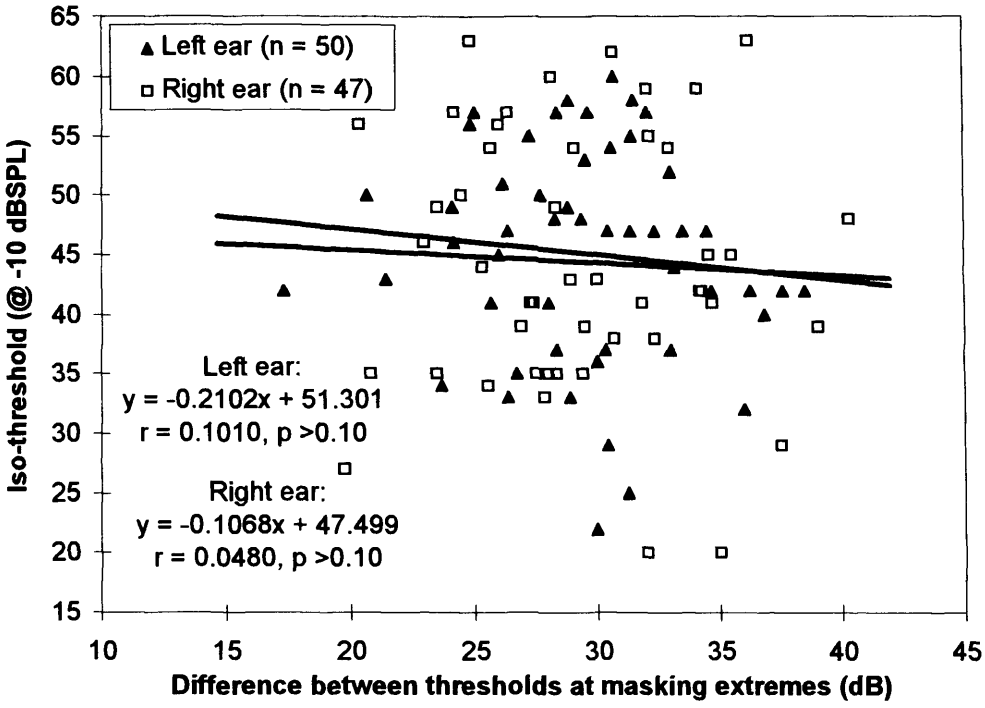


Fig. 3.9.2.2. Relationship between 4 kHz DPOAE growth rate iso-threshold and frequency resolution in controls

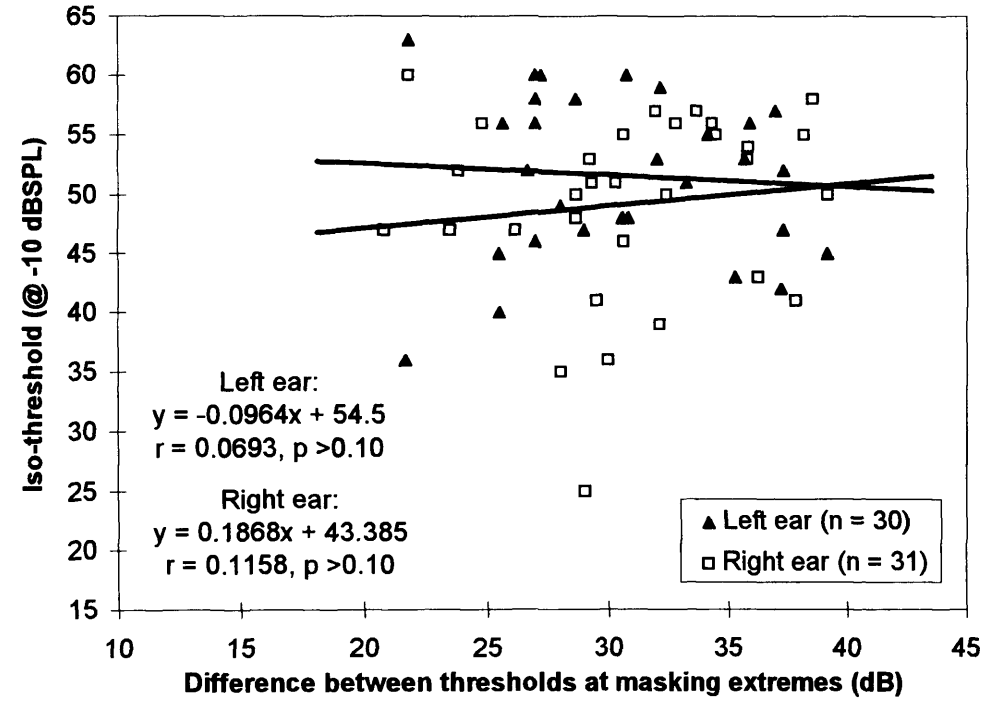


Fig. 3.9.2.3. Relationship between 8 kHz DPOAE growth rate iso-threshold and frequency resolution in controls

3.9.3. FREQUENCY RESOLUTION AND PTA

For 2, 4 and 8 kHz, the difference (dB) between masking extremes was found to correlate with pure tone threshold for both left and right ears ($p < 0.01$). The distributions suggest that those individuals with the most sensitive pure tone thresholds also have the most selective frequency resolution. In particular, at the highest frequency tested (8 kHz), a large proportion of the variability was accounted for by the correlation ($r = 0.74\text{-}0.83$; $p < 0.001$). Hence as the correlation was highly predictive at 8 kHz, the difference (dB) between masking extremes and pure tone threshold measures can not be assumed to be at all independent. Scatter plots and regression analyses are shown, for all three frequencies in Figures 3.9.3.1., 3.9.3.2. and 3.9.3.3. respectively.

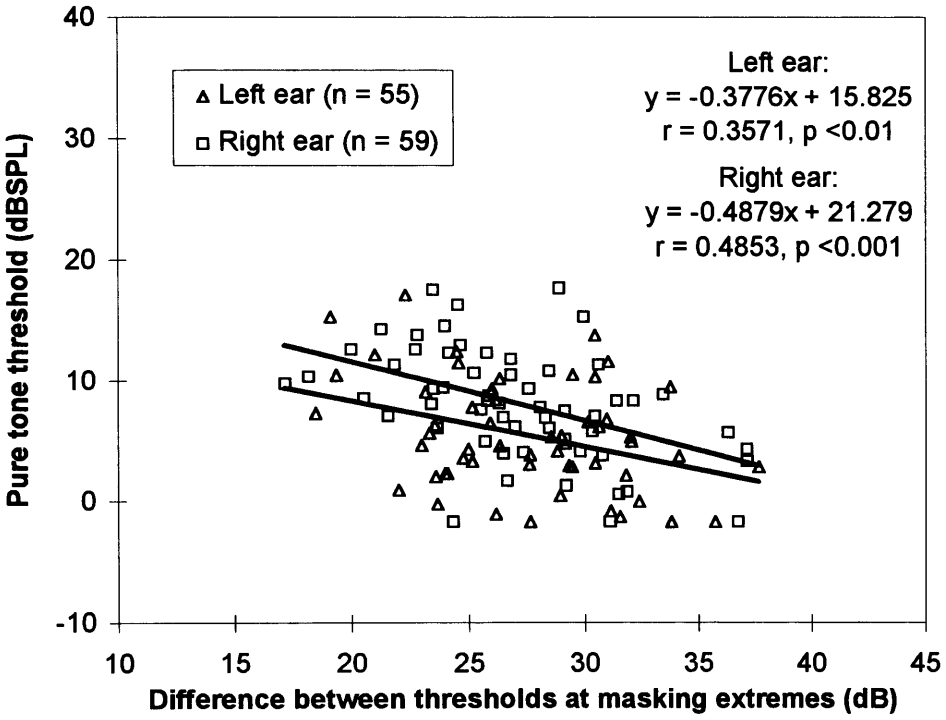


Fig. 3.9.3.1. Relationship between 2 kHz pure tone threshold and frequency resolution in controls

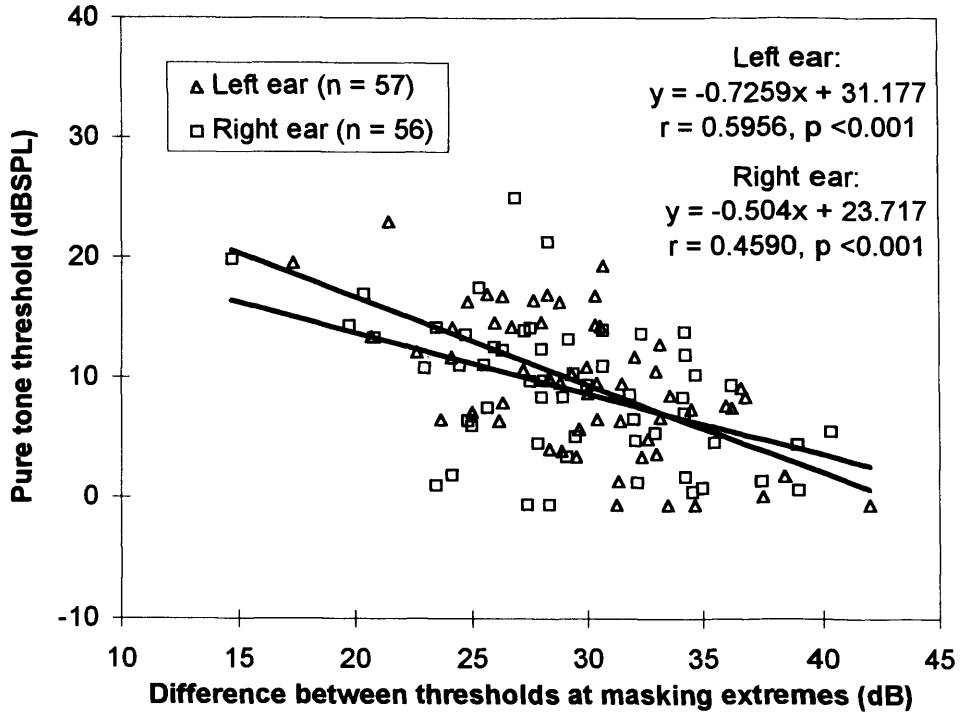


Fig. 3.9.3.2. Relationship between 4 kHz pure tone threshold and frequency resolution in controls

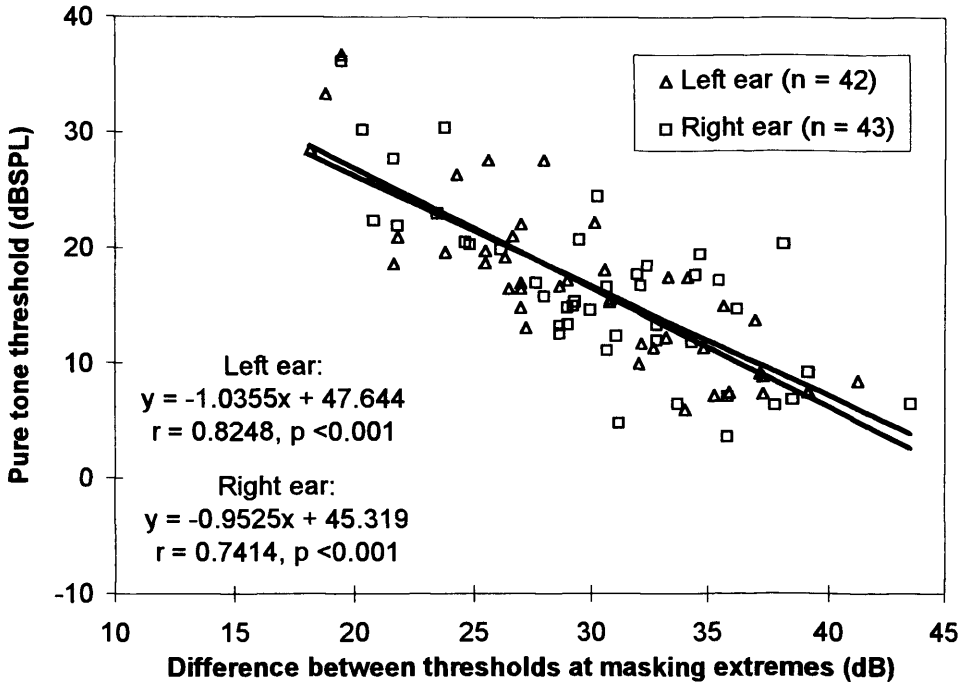


Fig. 3.9.3.3. Relationship between 8 kHz pure tone threshold and frequency resolution in controls

3.9.4. SOAEs AND CSSOAEs

An example of SOAEs and CSSOAEs recorded sequentially from the same ear is shown in Figure 3.9.4.1..

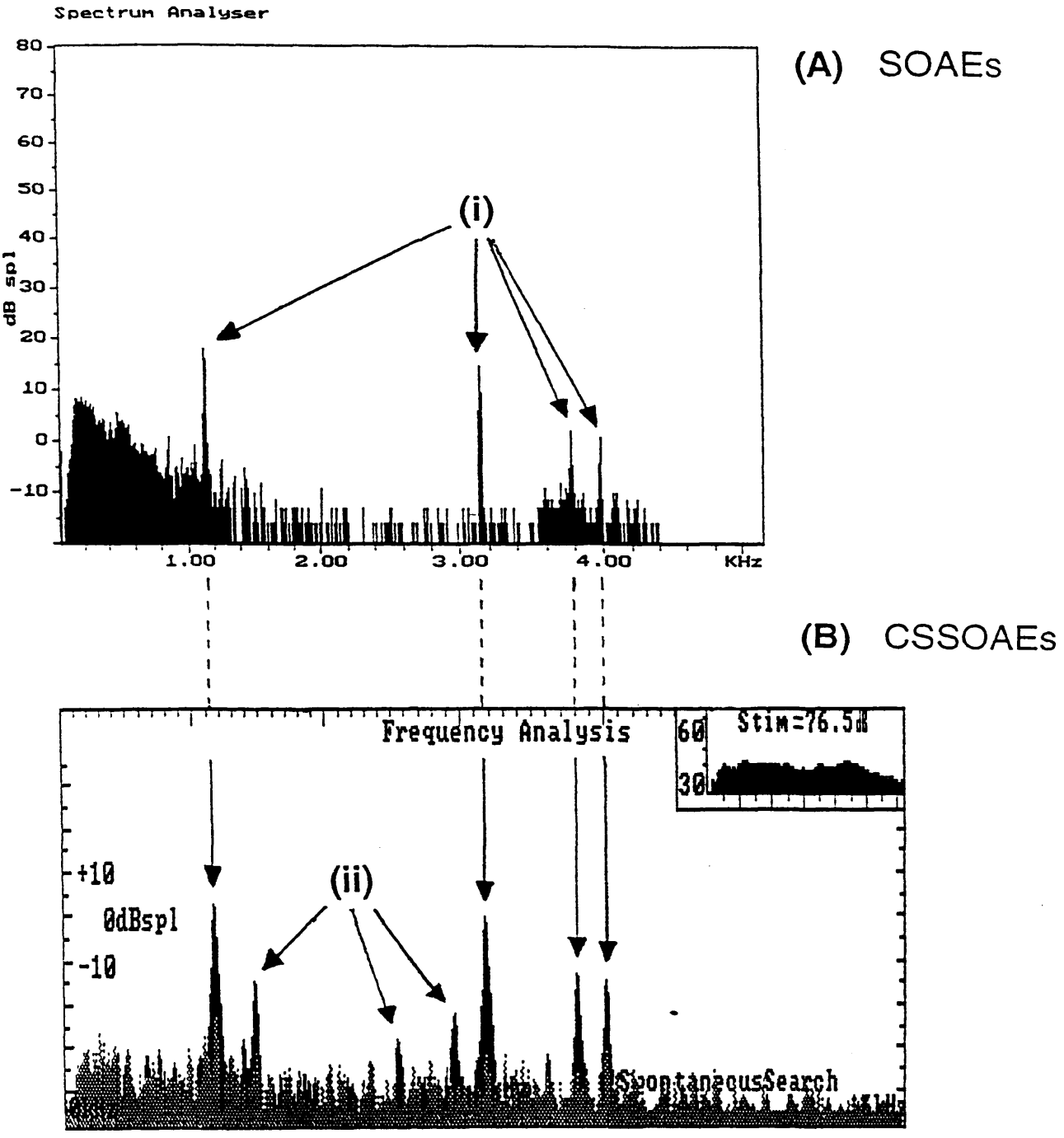


Fig. 3.9.4.1. **(A)** SOAEs recorded using the ILO92 'spectrum analyser' **(B)** CSSOAEs recorded using the ILO88 'synchronised spontaneous search'. For SOAEs and CSSOAEs measured in the same ear: **(i)** SOAEs above 7 dBSPL correspond with CSSOAEs at the same frequency, and are 14 dB greater in amplitude; and, **(ii)** CSSOAEs have a higher prevalence than SOAEs (see text overleaf).

Figure 3.9.4.2. shows a scatter plot of amplitude versus frequency, summarising the relationship between SOAEs and CSSOAEs (for left ears n = 65, and for right ears n = 66). There were significantly more ears with CSSOAEs than SOAEs for both left ears (40 with CSSOAE peaks and 15 with SOAE peaks; χ^2 = 19.73, df = 1, $p < 0.001$) and right ears (38 with CSSOAE peaks and 12 with SOAE peaks; χ^2 = 21.79, df = 1, $p < 0.001$). Overall, 60 % of ears had one or more CSSOAEs and 21 % of ears had one or more SOAEs. Also, the maximum number of peaks per ear was higher for CSSOAEs (n = 8) than SOAEs (n = 4). Approximately one-third more CSSOAE peaks were observed in right ears (n = 123) than left ears (n = 93), although the same was not true for SOAEs (24 peaks in right ears and 23 peaks in left ears). Overall, there were four-five fold more CSSOAE peaks than SOAE peaks.

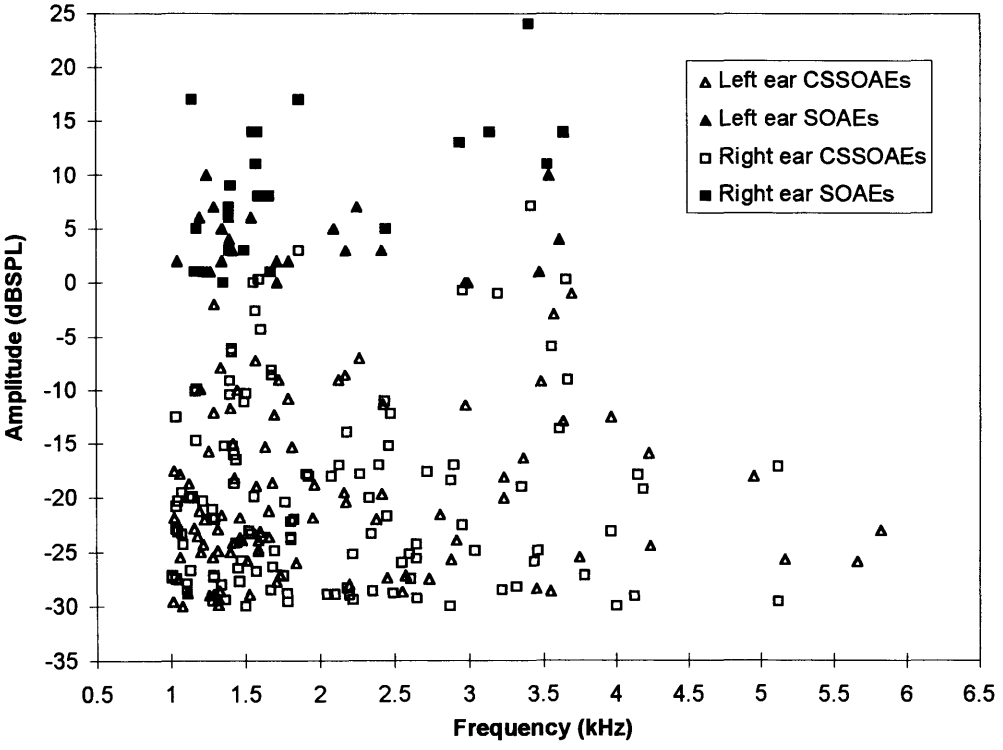


Fig. 3.9.4.2. Distribution of SOAEs and CSSOAEs in controls

The frequency of CSSOAEs (mean+/-SD: left ears = 2.09+/-1.10 kHz; right ears = 2.06+/-0.94 kHz) and SOAEs (mean+/-SD: left ears = 2.04+/-0.88 kHz; right ears = 1.91+/-0.83 kHz) were approximately equal (Mann-Whitney U tests, p = 0.88 and 0.53 respectively). The maximum frequencies were 5.82 kHz for a CSSOAE and 3.65 kHz for a SOAE.

The maximum amplitudes were 7.1 dBSPL for a CSSOAE and 24.0 dBSPL for a SOAE. The amplitude of CSSOAEs (mean+/-SD: left ears = -20.30+/-7.20 dBSPL; right ears = -19.90+/-8.70 dBSPL) was significantly lower than SOAEs (mean+/-SD: left ears = 4.08+/-3.60 dBSPL; right ears = 8.96+/-6.29 dBSPL; $p < 0.001$, Type III T and Mann-Whitney U tests respectively). For 94% of SOAEs there was a CSSOAE which corresponded in frequency, and for 20% of CSSOAEs there was a SOAE which corresponded in frequency (+/-12.5 Hz*). Furthermore, every SOAE above 7 dBSPL was associated with a CSSOAE, and every CSSOAE above -9 dBSPL was associated with a SOAE of equivalent frequency.

* Again, it should be remembered that all CSSOAE frequency values presented here are too high by 12.5 Hz due to an error in the ILO88 software (Smurzynski & Probst, 1996).

Figure 3.9.4.3. shows the relationship between SOAE and CSSOAE amplitude, for ears in which both were seen at the same frequency (left ear n = 21 and p <0.001; right ear n = 23 and p <0.001). The relationship was linear - at least over the approximate ranges of 0-25 dBSPL for SOAEs and -15-10 dBSPL for CSSOAEs - with the equivalent of a 14 dB offset.

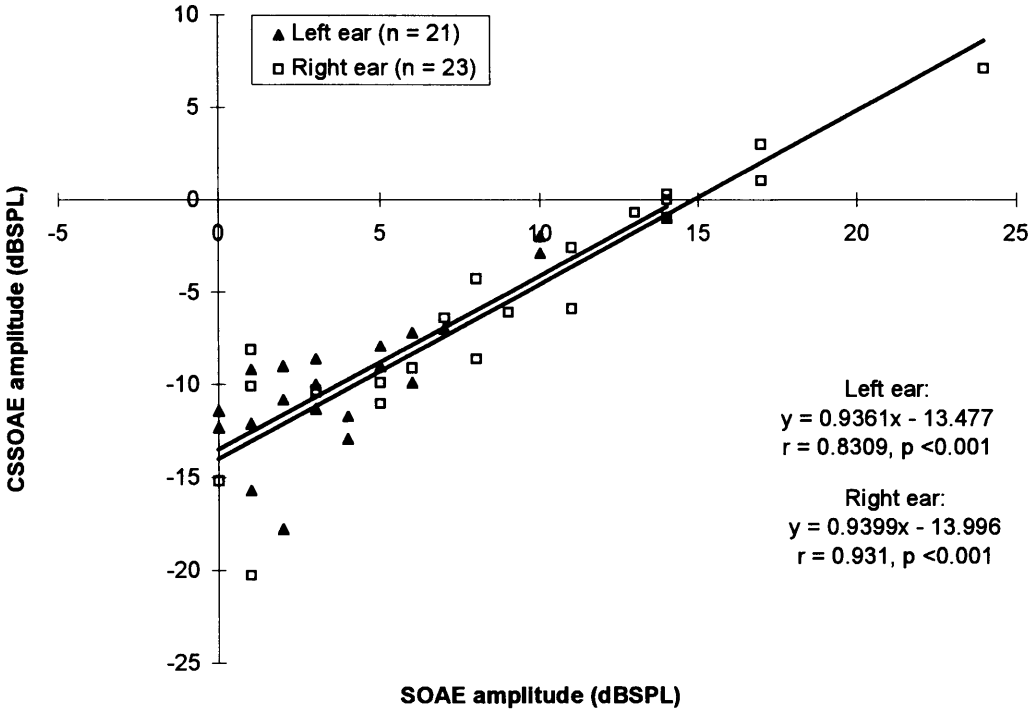


Fig. 3.9.4.3. Relationship between SOAE and CSSOAE amplitude (both in dBSPL) in controls

DISCUSSION

All tests are compared for: test-retest variability; effect of age; relationships between different tests; dose-response relationship; and, control versus patient performance. In conclusion, the findings from this mechanistic approach are summarised.

4.1. CONTROL VARIABILITY

4.1.1. INTRA-INDIVIDUAL VARIABILITY

To establish the stability of each test it is necessary to consider the intra-individual variability (over time). Table 4.1.1. shows the ranges of variability were similar for all the tests. The residual variation of test-retest measurements could be attributed to: external noise (e.g. computer fan); internal noise (e.g. body movements / posture, respiration, GI tract, blood flow); concentration; and, environmental preconditioning (e.g. TTS, PTS). A further important cause of intra-individual variation for psychophysical testing can be learning effects. Although there was no noticeable improvement in threshold estimates with repeated measurements in either PTA or frequency resolution.

For all OAE testing it is likely there would be an initialising effect, causing a small increase in OAE amplitude during the first minutes immediately following probe insertion (McFadden & Pasanen, 1994; possibly due to decreasing background noise causing an increase in 'gain'). However, any initialising effect was expected to be equal between control and patient groups. Furthermore, whenever possible OAE tests were performed in the same order to minimise any confounding initialising effect.

DPOAE input-output function shape was found to occasionally fluctuate between linear and flat-steep or flat-steep and diphasic. This somewhat complicates the use of shape as an indicator of cochlear

function. It could be assumed that changes in shape over time equally affect control and patient groups. However, particular shapes - or even iso-criteria - may be more susceptible to change than others, and the proportions of different shapes are not equal across groups (see Table 3.6.4.4.). Furthermore, aminoglycoside exposure itself may affect the type or degree of changes in shape over time.

TEST	MINIMUM AND MAXIMUM SDs	SDs AS PERCENTAGE OF MEANS
HIGH FREQUENCY PTA (at 10, 12, 14 and 16 kHz)	1.6 - 6.6 dB	4 - 38 %
FREQUENCY RESOLUTION (at 2, 4 and 8 kHz)	1.2 - 3.5 dB	4 - 18 %
DP-GRAMS (mean SD+/-SD)	1.26 - 1.39 dB +/- 0.47 - 0.60 dB	15 - 17 % +/- 37 - 43 %
DPOAE -10 dBSPL iso-criterion (at 2, 4, 6 and 8 kHz)	1.72 - 8.80 dB	4 - 20 %
DPOAE SLOPE (at 2, 4, 6 and 8 kHz)	0.06 - 0.52	6 - 18 %
SOAE AMPLITUDE	0.17 - 1.33 dB	2 - 64 %
SOAE-FREE SPECTRA (mean SD+/-SD)	1.26 - 1.53 dB +/- 1.09 - 1.19 dB	9 - 11 % +/- 78 - 87 %
CSSOAE AMPLITUDE	0.15 - 2.72 dB	3 - 252 %

TABLE 4.1.1. The overall* limits (minimum and maximum SD expressed as a percentage of mean values) of test-retest variability for different tests.**

* Pooling left and right ears.

** It is important to realise that when presented with a range of measurements - on the same scale and made with the same precision - the degree of error will be the same for each measurement, so as measurements decrease in size the degree of error will account for an increasing proportion of the total (c.f. 100 +/-1 vs. 1 +/-1).

The stability of long term test-retest variability measures - combined with calibration at both start and end of the study - confirm the absence of systematic errors entering measurements over time. On the whole, AM sessions were no less variable than PM sessions. Thus, expectations that AM sessions may be quieter and the subjects less fatigued than PM sessions were unfounded. Data has been collected for a more thorough evaluation of test-retest variability. In addition to the findings reported, ten sequential run-to-run measurements have been made for each of the objective tests, and all tests could be further analysed by random effect factors nested ANOVA. However, a more precise partitioning of the variance with time is beyond the intended ototoxicity focus of this particular study.

4.1.2. INTER-INDIVIDUAL VARIABILITY

To establish the stability of each test it is necessary to consider the inter-individual variability (between people), including any sex- and age-related effects. The test results of 11.3 % of control subjects were rejected completely due to failure to meet the screening criteria (3.8 % of which had unilateral sensorineural loss). It was of fundamental importance to thoroughly evaluate the results of the remaining 88.7 % of control subjects - independently of patients - in order to establish the range of what constitutes 'normal' (see Table 4.1.2. and following text). Auditory differences between normal individuals could be due to many factors, including the: central nervous system (with psychophysical testing); inner ear; middle ear transmission; and, ear canal dimensions.

TEST	MINIMUM AND MAXIMUM SDs	SDs AS PERCENTAGE OF MEANS
HIGH FREQUENCY PTA (at 10, 12, 14 and 16 kHz)	8.8 - 18.7 dB	30 - 42 %
FREQUENCY RESOLUTION (at 2, 4 and 8 kHz)	4.3 - 6.6 dB	16 - 22 %
DP-GRAMS (mean SD+/-SD)	5.77 - 5.88 dB +/- 0.62 - 0.68 dB	76 - 80 % +/- 11 - 12 %
DPOAE -10 dBSPL iso-criterion (at 2, 4, 6 and 8 kHz)	7.28 - 10.49 dB	14 - 24 %
DPOAE SLOPE (at 2, 4, 6 and 8 kHz)	0.24 - 0.36	25 - 39 %
SOAE AMPLITUDE	3.57 - 6.29 dB	70 - 88 %
SOAE-FREE SPECTRA (mean SD+/-SD)	1.51 - 1.52 dB +/- 1.25 - 1.33 dB	10 - 11 % +/- 82 - 88 %
CSSOAE AMPLITUDE	7.13 - 8.67 dB	35 - 39 %

TABLE 4.1.2. The overall limits (minimum and maximum SD expressed as a percentage of mean values) of control variability for different tests.

For high frequency PTA, the overall median normal threshold values found in control subjects were: 20 dBSPL at 10 kHz, 30 dBSPL at 12 kHz, 40 dBSPL at 14 kHz, and 60 dBSPL at 16 kHz (measured with Etymotic insert earphones to the nearest 5 dB). All of these thresholds are similar (worse by 0-30 dB, dissociating with increasing frequency) to published best threshold estimates (reviewed by Northern & Ratkiewicz, 1985). For other tests, the range of mean normal values found in control subjects can also be expected to vary with test frequency. However, pooling information across frequencies allows easier comparison with published values.

For frequency resolution, mean differences between thresholds at masking extremes were 27.3-30.5 dB. At 2 kHz, Patterson (1976) found an average difference of about 30 dB (n = 4 individuals, ages unspecified). At 4 kHz, Bergman et al. (1992) found differences of 10-25 dB (n = 44 ears, age = 22-39 years; although Zhou (1995) did find differences of about 30 dB (n = 3 individuals with an average age of 21 years). Whilst at 8 kHz, Shailer et al. (1990) found differences of about 35 dB (n = 3 individuals, age = 21-48 years). The spectrum levels in the above studies were approximately comparable at 40-50 dBSPL/Hz.

Mean DP-grams (see Figures 3.5.4.A. and B.) were in close agreement with published normal plots (Vinck et al., 1996; n = 101 ears, age = 19-28 years). For DPOAE input-output functions, mean -10 dBSPL iso-criteria were 36.97-50.61 dBSPL, mean slopes were 0.84-1.05, and overall 50-76 % of shapes were linear. Very similar ranges have been widely shown for -10 dBSPL iso-criteria and slopes (Lonsbury-Martin et al., 1991, n = 20 ears from 31-40 years old; Ohlms et al., 1991, n = 44 ears of unspecified age; Vinck et al., 1996, n = 101 ears as above). Less information is available on shape, but Nelson and Kimberley (1992) reported 9-31 % of shapes were linear at 2 and 4 kHz (n = 32, ages unspecified). The discrepancy in linear shapes is accounted for by a higher proportion of diphasic and saturating shapes, and could be due to differences in designation criteria or older subjects.

SOAE characteristics are very much dependent on the designation criteria and so are difficult to compare across studies (see methods 2.6.3.). Thus for the criteria adopted, 18-23 % of ears had one or more SOAEs, there were 0.35-0.37 SOAEs per ear*, and mean amplitudes were 4.08-8.96 dBSPL. For CSSOAEs, 58-60 % of ears had one or more CSSOAEs, there were 1.41-1.86 CSSOAEs per ear*, and mean amplitudes were -20.02 to -20.32 dBSPL. A very similar CSSOAE

* Including both emitting and non-emitting ears.

occurrence and mean amplitude has been reported by Prieve & Falter (1995; n = 41 ears, age = 19-29 years).

There are numerous factors that may influence inter-individual test variability, and these are now considered specifically for each test in turn. For psychophysical measurements, the information and instructions given to subjects was standardised, and co-workers often observed testing to ensure the operator's technique appeared consistent and involved no prompting (intentional or otherwise). The two ears of one individual may have different frequency resolution due to differences in noise exposure (e.g. occupational) or training (e.g. playing of asymmetrical musical instruments). There is also the possibility of masking interference by SOAEs, if coincident with the centre frequency of interest. Furthermore, differences in the proportion of false responses between individuals may reflect differences in internal response criteria and bias threshold measurements. Hence, a low proportion of false responses may indicate a threshold estimate is worse than in reality, as the subject is waiting for more certainty. Finally, intra-individual consistency in frequency resolution thresholds indicates a single estimate could be a more efficient use of test time than a 6 point estimate of threshold for each masking condition.

For DPOAE input-output function measurements, the designation of shape and extrapolation of iso-criteria can be extremely subjective; in the future wherever possible such criteria should be implemented 'blind'. In addition, linear input-output functions were more common than saturating ones, indicating that the stimulus intensity could have been inadequate to show saturation. However, it is likely that cochlear responses become passive above 70 dBSPL (Lonsbury-Martin et al., 1990a).

For SOAE and CSSOAE measurements, it is important to realise the distinction between the number of ears with peaks and the number of peaks in ears, although the two are obviously linked. Previous reports of an unexplained higher occurrence of SOAEs in right ears than left ears

(e.g. Penner et al., 1993) were not completely supported. SOAEs were found in equal proportions in both ears, but tended to have higher amplitudes in right ears compared with left ears. However, CSSOAEs tended to have equal amplitudes in both ears, but there were a third more CSSOAEs found in right ears compared with left ears (but not more ears with CSSOAEs). Hence for CSSOAEs, there would appear to be more right than left ear multiple emitters. Subtle differences between SOAEs and CSSOAEs suggest different contributions by OHCs to active cochlear processing by right and left ears, which requires further investigation. The idiosyncratic SOAE and CSSOAE right-left asymmetry - revealing a dominant side - may be similar in origin to 'handedness' and 'eyeness' (thus 'earness' would obviously be dependent on genetic and / or environmental factors). There were also twice as many female ears with SOAEs and CSSOAEs compared with male ears (and more SOAEs and CSSOAEs in female ears; conforming closely with published work relating SOAEs to gender (Whitehead et al., 1989). The higher occurrence of SOAEs in females (also confounded by race; Whitehead et al., 1993) is also suggestive of a genetic, or hormonal effect on hearing.

For certain tests it was necessary to partition groups according to age (into approximately equal group sizes, which conveniently corresponded to recruitment site) before undertaking subsequent comparative analyses. Over the age ranges tested, the only significant age-related findings ($p < 0.05$) were with high frequency pure tone thresholds at 10, 12, 14 and 16 kHz, and with mean difference for frequency resolution at 4 and 8 kHz. Thus for these tests only, control subjects and patients were further split into juvenile and adult groups before comparison. However, it should be remembered that there were no obvious systematic differences in treatment regimes between hospital sites; and that in any case, a site-specific measure would be of little widespread clinical use. Of course, partitioning the groups unnecessarily would also reduce the statistical power. Therefore, the results from other tests were not partitioned according to age.

4.1.3. IMPLICATIONS AND RECOMMENDATIONS

For all tests variability has followed the pattern: Inter-individual > Intra-individual (revealed by comparing Tables 4.1.1. and 4.1.2.). Therefore, it should be easier to distinguish differences by following the same ear over time, than comparing against a normative group. Furthermore, with increasing frequency, psychophysical test variability appears to decrease within an individual, but increase between individuals (inferred from results 3.3. and 3.4.). However, a more precise partitioning of the variance with frequency is again beyond the intended ototoxicity focus of this particular study. Nevertheless, the extensive normative database itself is a valuable resource for other clinical studies employing these techniques. In addition, there is an opportunity here to investigate the relationships between different measures of auditory function (particularly DPOAEs vs. tympanometry, DPOAEs vs. frequency resolution, frequency resolution vs. PTA; and, SOAEs vs. CSSOAEs). However, such cross-test correlation within the control data also detract from the intended focus of the study and so will only be considered briefly.

4.2. RELATIONSHIPS BETWEEN TESTS

4.2.1. TYMPANOMETRY, DPOAES AND FREQUENCY RESOLUTION

Overall for control subjects, DPOAE -10 dBSPL iso-criterion* was found to be independent of tympanometry compliance, peak pressure and frequency resolution. The majority of the tympanometry data (see results 3.1.4., and Figures 3.9.1.1-8.) was within the accepted normal ranges (see methods 2.2.1.). Therefore, the normal range of middle ear function (as measured by compliance and peak pressure at 226 Hz) does not appear to affect DPOAEs (as measured by iso-criteria at 2, 4, 6, and 8 kHz). Although much work has been carried out demonstrating the adverse effect of extreme middle ear pressure on OAEs (Margolis & Trine, 1997), there appears to be comparatively little available information on the effect of extreme middle ear compliance, or variations within the normal ranges of middle ear function. Lonsbury-Martin et al. (1990b) did assess the effect of normal middle ear compliance and peak pressure (and at the DPOAE frequency under test by using multi-frequency tympanometry); but similarly found no correlation and recommended the use of more sensitive tympanometry. Therefore, future research in to the effects of normal middle ear function on OAEs still has the potential improve the specificity of OAE measurements.

Similarly, frequency resolution was found to be independent of DPOAE -10 dBSPL iso-criterion at 2, 4 and 8 kHz. Therefore providing a basis for measuring changes in selectivity and OHC function separately. However, frequency resolution was found to be dependent on PTA at 2, 4 and 8 kHz. Especially at 8 kHz, where frequency resolution and PTA had a highly predictive relationship ($p < 0.001$ and $r = 0.74$-0.83), indicating that selectivity was certainly not being measured independently of sensitivity at the highest test frequency. It is possible that the

* DPOAE iso-criterion (at -10 dBSPL), slope and maximum threshold (at 70 dBSPL) are interrelated and any difference in one may be reflected in the other two.

masking level was insufficient to reduce the contribution from sensitivity to frequency resolution (although greater amplification was not possible with the available equipment due to distortion). Thus, there was no advantage in assessing frequency resolution at 8 kHz as PTA at 8 kHz could act as an effective surrogate measure. In any case, as both control subjects and patients passed screening criteria that included PTA assessment at 8 kHz, there was little - if any - possibility of detecting a sub-clinical difference in frequency resolution at 8 kHz.

4.2.2. SOAES AND CSSOAES

The CSSOAE and SOAE methods have been compared in audiologically normal individuals and differences have been identified. The CSSOAE method was found to: increase the total number of recordable discrete frequency emissions (by four-five fold); increase the number of emitting ears (by three-fold; $p < 0.001$); and, decrease the amplitude of emissions coincident in frequency (by 14 dB; $p < 0.001$ and $r = 0.83\text{-}0.93$). The larger number of CSSOAEs may be attributed to a residual transient-evoked component and lowering of the noise floor, whereas the lower amplitude of CSSOAEs may be due to an insufficient recovery time for SOAEs to achieve pre-stimulus levels. SOAEs would conceptually appear to be the result of a defective electromechanical amplification process which is less stable and / or over-active in frequency-specific regions. A larger increase in the number of peaks than in the number of emitting ears, reveals that multiple peaks must also be more common with the CSSOAE method. Thus, a higher proportion of multiple emitters may be due to the CSSOAE method enhancing cochlear instability in predisposed individuals.

Study of the correlation between SOAEs and CSSOAEs in occurrence, frequency (Hz) and amplitude has revitalised an area for investigation in audiological science that may reveal a greater insight into cochlear processes. Augmentation of SOAEs by the CSSOAE method presents the possibility of further study (particularly in characterising the

intensity-response and time-course) of the mechanisms underlying OAE generation. Indeed, the synchronisation of SOAEs to tones - with the effects of aspirin - has already provided insight into the suppression of oscillation and generation of emissions (Long et al., 1991). Furthermore, synchronisation of SOAEs to clicks over a range of intervals has revealed the influence of phase on re-synchronisation of long-lasting CSSOAEs (Gobsch & Tietze, 1993). Intuitively, changing the stimulus level of the CSSOAE method could separate SOAEs from long-lasting TEOAE components. However, preliminary findings suggest that click amplitude is approximately proportional to peak amplitude for all CSSOAEs independent of the presence of coexisting SOAEs (Burr, unpublished work). Much more detailed work is necessary to follow the changing characteristics of CSSOAEs as they decay with time to elucidate the transfer from evoked to non-evoked OAEs.

4.3. PATIENTS AND DOSE-RESPONSE

4.3.1. INCLUSION OF PATIENTS

Subjects who considered the tests to be important (for themselves or others) may exert a higher degree of concentration. Increasing age and suspected hearing problems may increase compliance and therefore bias inclusion. Conversely, some patients rationalise CF as terminal and so may not consider care for hearing protection a high priority. Similarly, a sub-clinical problem should have no bearing on inclusion. Thus, health-conscious individuals are more likely to volunteer for medical research. These people can be considered to fall into two main categories: individuals who look after their health, and are consequently more healthy than 'normal'; and, individuals who suspect a problem, and are consequently less healthy. As the two groups complement each other, it is possible that they might balance each other out. Nevertheless, high dose patients are more likely to be too ill to test and therefore be under-represented.

4.3.2. PATIENT MEDICAL RECORDS

The patients are not a homogeneous group with respect to availability of information on therapy. A full audit of patients' medical records reveals inconsistencies and gaps in the documentation of therapy. Conversations with medical staff suggest that alterations to therapy are not always documented and internal evidence of omissions is common. The intravenous administration of aminoglycosides varies in: degree of dilution (sometimes completely undiluted); rapid bolus or drip (over 1-30 minutes, usually more variable with children); administration frequency (1-3 doses per day); numerous different co-administered drugs (typically 20+, e.g. ceftazidime); dose/kg; peak and trough serum levels; duration of course; number of courses; duration between courses; and, other pre-disposing risk factor(s) (including existing hearing problems and drug sensitivity or allergy). Instances of low aminoglycoside exposure in

patients may be due to: younger age; lower severity of symptoms; or because physicians avoid use. Adult patients are more likely to have missing records because of attending multiple hospitals. Where records are missing, past aminoglycoside exposure has to be estimated from patient questioning. Not all self-reported courses will be of aminoglycosides (as patients confuse aminoglycosides with other intravenous antibiotics), and not all courses of aminoglycoside will be remembered. Furthermore, patient compliance determines accuracy of records on home therapy, and answers to questioning include admissions of non-compliance. Patients also report that serum levels were often not checked when children (as care was often at a hospital without a specialised centre). The patients recruited included individuals with: depression; insulin overdose; heroin addiction; pregnancy; warfarin anticoagulant therapy (for protein S deficiency); renal sarcoidosis; heart-lung transplantation; sinus problems; grommets; test-induced nausea; epilepsy; neurofibromatosis; cerebral palsy; suspected meningitis; uncooperative behaviour; aminoglycoside nebulizers; simultaneous multiple aminoglycoside therapy; and, different cystic fibrosis genotypes. Therefore, it is doubtful that a more thorough evaluation of patients' histories would reveal any important differences between those exhibiting signs of ototoxicity and those who do not.

4.3.3. MEASUREMENT OF DOSE

Measurement of total dose (in days) was found to correlate with patient age ($p < 0.01$ and $r = 0.37$), although the variability increased dramatically above 20 years of age (Fig. 3.2.5.). This correlation will be confounded by: age of treatment onset; number of hospitals attended increasing with age; and, decreasing reliability of reported exposure with increasing age (compounded by missing medical records). Measures of internal dose will be confounded by: different hospital regimes; younger patients tending to be exposed to gentamicin and older exposed to tobramycin; and, prescribing difficulty with lower bodyweights.

Measures of external dose will be confounded by interpolation of doses and bodyweights.

In theory, prediction of the required dose to achieve a desired serum level should be straightforward. There is no absorption phase with intravenous administration, protein binding is negligible in the distribution phase, there is effectively no metabolism of aminoglycosides, and the risk of any laboratory analytical error is thought to be negligible although several potential problems have been suggested (De-Groot & Smith, 1987). However, serum sampling errors, differences in drug distribution, and highly variable excretion rates, all conspire to undermine reproducible prescription practice.

Serum sampling errors are primarily concerned with contamination and timing. Contamination with the administered dose may occur due to sampling from same site (where there may be some pooling of blood), or even sampling from the same portacath (where there may be some binding to plastic) as is common when intravenous access is poor. Also, aminoglycosides are thought to interact synergistically with β-lactam antibiotics (such as ampicillin, azlocillin, aztreonam, ceftazidime, flucloxacillin, imipenem and piperacillin) which are frequently concomitantly administered (Greenwood, 1992). Overall, aminoglycoside pharmacokinetics have established a distribution time of 5-60 minutes before serum levels peak following intravenous administration (reviewed by Siber et al., 1975). Therefore, there will be considerable variability in serum level measurements depending on chosen sampling time (ranging from 30 to 60 minutes) and dose level. Also, samples taken after infusions may be timed from the start or end of drug delivery. Furthermore, there will undoubtedly be deviations from therapeutic protocols (which themselves may vary between hospitals and over time).

Differences in distribution can occur irrespective of bodyweight, when the proportion of volume available for drug dispersion varies with state of hydration. Plasma clearance and volume of distribution for tobramycin and gentamicin are both higher in CF patients than non-CF

patients. The higher plasma clearance of gentamicin is accounted for by higher renal clearance, whereas the higher plasma clearance of tobramycin is not due to increased renal clearance and may be due to biliary excretion or hepatic induction. The available volume for drug distribution in CF patients may be greater due to both weight loss (because of a relative lack of adipose tissue) and hypervolaemia (because of increasing pulmonary disease). In addition, plasma clearance and volume of distribution both tend to be higher with tobramycin than gentamicin, although not significantly so (reviewed by De-Groot & Smith, 1987). So for the same therapeutic protocol (see introduction 1.6.3.) tobramycin may be expected to be less toxic than gentamicin.

Aminoglycosides are actively excreted by the kidney, with 80-90 % filtered at the glomerulus and 10-20 % reabsorbed at the proximal renal tubule (De-Groot & Smith, 1987). Overall, aminoglycoside pharmacokinetics have established an elimination half life of 1.2-4.4 hours following intravenous administration (reviewed by De-Groot & Smith, 1987). Excretion can vary widely with a multitude of factors, such as: infection; trauma; age (irrespective of bodyweight); and, co-administration of diuretics. High trough serum levels are hazardous and have for a long time been correlated with aminoglycoside toxicity (Barza & Lauermann, 1978). However, whether high trough serum levels are a cause or effect of nephrotoxicity is unclear. On the other hand, peak serum levels appeared to fluctuate more than trough serum levels (see results 3.2.3.) and so perhaps present more scope for investigating a correlation with ototoxicity (especially when elimination from the cochlea is slow; see introduction 1.6.6.). Nevertheless, for both peak and trough levels neither maximum value nor number of times exceeding the therapeutic range appeared to correlate with sensorineural loss.

In short, the original clinical rationale for measuring serum levels is the unpredictability of the relationship with administered dose - even in a controlled setting - primarily due to wide fluctuations in renal function (Kaye et al., 1974; Barza & Lauermann, 1978). Whether individually or

as a group, patients appear to be heterogeneous with respect to correlating external with internal dose, posing problems that prevent initial aminoglycoside prescriptions being either accurate or precise. Essentially, all the dose-related findings presented here (results 3.2.) are based on audit and not a controlled experimental design. Thus, the practical application of current therapeutic regimes is found to be fraught with difficulties.

4.3.4. ESTIMATION OF EXPOSURE

In order to determine the predictive value of dose-response plots, it was necessary to determine any relationship between the available external measures of total dose (e.g. days, grams) and the internal measure of dose (mg/l). There were significant correlations between internal and external dose for both gentamicin and tobramycin (Figures 3.2.2.1-2.). The relationships were stronger between different individuals ($p < 0.05$ and $r = 0.35$, $p < 0.001$ and $r = 0.56$ respectively) than within the same individual ($p < 0.02$ and $r = 0.62$, $p < 0.10$ and $r = 0.31$ respectively). This implies less benefit in trying to establish a dose-response by monitoring the same individuals over time, and supports comparing results from groups of individuals. However, there was still no observable dose-response relationship with any auditory test ($p > 0.05$). The range of dose employed can fluctuate between individuals for a number of reasons. In short, an individual's initial treatment is based firstly on weight and then modified using clinical experience to achieve the desired range of serum levels, and that dosage is then repeated until serum level indicates moderation. Thus, final external dosage can vary considerably between different individuals of the same weight in order to achieve a similar internal dosage.

Dose can be expressed as the total number of treatment days, for which a known proportion of serum levels were within the desired range of 5-12 mg/l. 86.7 % of known serum level measurements were within the desired range, although only 63.7 % of expected measures were

known. Due to the incomplete nature of medical records it was not possible to correlate any hearing losses observed with information concerning the: total dose; duration of therapy; and, mean or maximum peak serum levels. No correlation with internal dose could be observed because of the incomplete and isolated nature of serum level measures. Furthermore, aminoglycoside ototoxicity may require an internal dose to be maintained for a specific duration. It is unknown whether damage occurs with either a single high exposure, or repeated lower exposures and may depend on cumulative total dose or total above a certain threshold. The possibility remains that aminoglycoside ototoxicity could be a once only, or even a first-time exposure effect. A single detrimental exposure could easily be missed when only a few measurements of internal dose are made with each course of therapy. In addition, one or more risk factors may need to be present in order to predispose an individual to ototoxicity (e.g. compromised renal excretion; Jackson & Arcieri, 1971). Therefore, there are a large number of unforeseen complex interactions involving both pharmacokinetics and pharmacodynamics, which prevent the establishment of a dose-response relationship.

4.3.5. RELATION TO EXISTING RESEARCH

Existing publications (e.g. Crifo et al., 1980; Forman-Franco et al., 1979; Li et al., 1991; Morgan et al., 1988; Mulherin et al., 1991; Pedersen et al., 1987; Wood et al., 1996) do not comment on CF patients who have not been exposed to aminoglycoside therapy, or on the consequences of a long period without such exposure on the outcome of ototoxicity index measures. Amongst the many other aforementioned problems that may contribute to differences in the reported incidence and prevalence of ototoxicity in CF patients are: referral selection bias; differing PTA loss criteria; unrecognised sinus problems associated with chest infections; and, exclusion of patients who failed the screening criteria in the calculation of proportion suffering

loss. Patient selection may be unrepresentative due to: dose (confounded by age); locality (to minimise travel cost, inconvenience and increased chance of complete medical records); compliant behaviour; fitness to test; and, hospital captivity (complicated by those who are more susceptible to infections). Furthermore, psychophysical performance (PTA) has been demonstrated to decrease with increasing non-hearing related illness (Davey et al., 1982).

4.3.6. IMPLICATIONS AND RECOMMENDATIONS

Sensorineural losses should - when possible - be correlated with history, especially excessive noise exposure, as this may explain anomalous cases of hearing deficit associated with low aminoglycoside exposure. However, reports of lifetime noise exposure are susceptible to memory recall and difficult to both qualify and quantify by questionnaire. Thus, it was considered reasonable to assume noise exposure between patient and control groups was equal. Also, serendipitous reports of tinnitus during infusions could have been investigated by using a more thorough questionnaire.

The accurate measurement of aminoglycoside exposure remains a substantial problem. The total number of courses is dependent on courses - or rather infections - of different duration (i.e. continuity of exposure varies). Also, the number of total days exposure is dependent on varying doses (i.e. low versus high dose exposure on different days). Finally, the number of total grams is dependent on bodyweight (and neither relate to internal exposure). Obviously, any findings regarding the relationship between serum level and side-effect would be of clinical use. However, serum level measurements are extremely sparse (at best a paired trough and peak level for each course and change of dose).

This research has been unable to demonstrate the dose-response relationship necessary to confirm the occurrence of aminoglycoside ototoxicity. However, patients with sensorineural loss were exposed to significantly more total days treatment than patients passing the PTA

criteria (see results 3.2.4.). Furthermore, all the patients with confirmed deficits do show a pattern of high frequency sensorineural hearing loss that is indicative of ototoxicity (Figures 3.1.5.1-3.). Clear signs of ototoxicity are needed to justify the use of drugs that are less toxic but more expensive (e.g. merepenem, imipenem). As a consequence of the availability of such signs, patients with previously unrecognised deficits have had their therapy changed as a direct consequence of being involved in this study.

4.4. COMPARISON OF CONTROLS VERSUS PATIENTS

4.4.1. STANDARD PTA

The test results of 11.3 % of control subjects and 10.3 % of patients were rejected completely due to failure to meet the screening criteria. A further 11.5 % of screened patients had sensorineural loss (3.9 % of which were unilateral). If patients with unilateral PTA loss were excluded on the premise that a similar proportion of control subjects exhibited such losses (3.8 %; which were of unknown origin), then 7.7 % of patients exhibited signs of ototoxicity. The observed proportion of CF patients exhibiting a significant loss in PTA is within the expected values of tobramycin (6.1 %) and gentamicin (8.3 %) ototoxicity in non-CF patients (1895 patients from 28 trials and 572 patients from 14 trials respectively; reviewed by Kahlmeter & Dahlager, 1984). One report of 42 adult CF patients carefully monitored by PTA revealed no cases of tobramycin ototoxicity (Li et al., 1991). Whereas at the other extreme, in non-CF patients the highest known incidence of gentamicin ototoxicity is 45% (Winkel et al., 1978). Nevertheless, for the regularly repeated courses of therapy received by CF patients throughout their lives, a higher degree of ototoxicity might be expected (closer to 30 %; Morgan et al., 1988). Here (results 3.1.5.), sensorineural losses were found in 5.1 % of paediatric and 18.0 % of adult patients (an approximate ratio of 1:3); the difference is not significant ($p > 0.05$) but is indicative of an age, hospital site, and / or dose related effect.

Reports of up to a ten-fold discrepancy in ototoxic incidence between different studies of the same aminoglycoside have long been lamented (Fee, 1980), and there are a number of practical factors that still prevent a consensus. Of course it is likely that 'negative' results (reporting low or no ototoxicity) are underrepresented in the literature. It

is also important to realise that incidence and prevalence* can be confused in the literature (e.g. Forman-Franco et al., 1979) and that this error can lead to the misguided assumption that quoted percentages are control-adjusted when they are not. Arguably the most thorough review of aminoglycoside ototoxicity in non-CF patients (Kahlmeter & Dahlager, 1984), does not appear to have taken account of the inherent difference due to incidence and prevalence across different reports. A further flaw concerns the identification of ototoxicity. Studies have employed widely different criteria as to what constitutes an ototoxic incident. Common PTA prerequisites are that: 1 or even 2 or more frequencies (e.g. Davey et al., 1982 and Li et al., 1991 respectively) exceed a limit of 25-30 dBHL (e.g. Forman-Franco et al., 1979 and Mulherin et al., 1991 respectively); or, exhibit a change of 10-20 dB (e.g. Tange et al., 1995 and Davey et al., 1982 respectively); at the highest test frequencies, or any frequency (e.g. Mulherin et al., 1991 and Wood et al., 1996 respectively); and, these requirements may be imposed unilaterally or bilaterally (e.g. Pedersen et al., 1987 and Morgan et al., 1988 respectively). A further complicating factor is that criteria are often applied differentially, e.g. a change of 20 dB or more at one frequency and 15 dB or more at two adjacent frequencies (e.g. Wood et al., 1996). In addition, many of the above studies did not specify which or how many frequencies were tested (e.g. the apparently tightly controlled randomised trial by Tange et al. (1995) implies criteria were applied equally for 'conventional' and 'high frequency audiometry'). Others have not specified any criteria at all, only reporting the threshold values that failed (Meyers, 1970), or even designating loss simply as 'sensorineural' (Crifo et al., 1980).

* Incidence is the number of new cases occurring, whereas prevalence is the number of existing cases. Also remember values should be per person, not per ear.

4.4.2. DIFFERENCES BETWEEN CONTROL SUBJECTS AND PATIENTS

Sub-clinical effects were investigated by excluding patients who failed the standard PTA criteria and comparing with identically screened control subjects. However, more profound effects could only become apparent when also including patients with bilateral sensorineural loss. Although no control subjects exhibited bilateral sensorineural loss, there are a number of complications associated with including patients with such losses (see methods 2.6.3.). When included for comparison with control subjects, patients with bilateral sensorineural loss will contribute a varying proportion of the overall total of patients when considering different tests. Comparisons including patients with bilateral sensorineural loss will be further confounded by the varying gradations of loss. All comparisons will be confounded by the varying degrees of dilution of 'unaffected' patients by 'affected' patients. Furthermore, low dose patients will dilute the reporting of any effects, especially if only a small proportion of high dose patients are affected (with the remainder of patients being equivalent to control subjects).

4.4.3. HIGH FREQUENCY PTA

For high frequency PTA, sample size was in most cases sufficient to detect any difference between patient and control groups of 11 dB or more in threshold. Consistent significant differences ($p > 0.05$) in mean threshold - affecting consecutive frequencies, or both left and right ears - were not apparent, even when including patients with bilateral sensorineural loss. Whereas, significant differences in variance were greatest at the lower frequencies of 10 kHz and 12 kHz ($p < 0.001$), and decreased with increasing frequency (14 kHz $p = 0.05$-0.005), with there being no significant difference in variance at 16 kHz ($p > 0.05$). Those patients with sensorineural loss were dissociated from the control subjects and other patients (Figures 3.3.3.1-4.A and B.). The gap in threshold between 'normal' and 'abnormal' is greatest at 10 kHz and decreases with increasing frequency. Both the average threshold and

similarity in variance increase with increasing frequency (see Table 3.3.4.). So with increasing frequency, differences in threshold variability decrease equivalent to decreases in threshold dissociation. Hence, high frequency PTA becomes a poorer indicator of toxicity further away from the frequency (8 kHz) at which loss was confirmed. This finding is consistent with the work of Fausti et al. (1992) who proposed 8-14 kHz to be the most suitable frequency range for detecting ototoxicity. Furthermore, there appears to be an uppermost limit for responses of 95-100 dBSPL at all high frequencies, suggesting a saturation in loss of function that is independent of frequency. High frequency thresholds plateau before reaching the limits of the test equipment, in both control subjects and patients with sensorineural loss. There may be a limit to the extent of structural damage that can be caused by aminoglycoside exposure at the doses employed. However, the maximum functional range is most likely explained by substantial recruitment effects at high intensities. High frequency PTA would therefore appear to be limited in usefulness to a 95-100 dBSPL response ceiling.

4.4.4. FREQUENCY RESOLUTION

For frequency resolution, sample size was in most cases sufficient to detect any change between patient and control groups of 5 dB or more in difference between masking extremes. Extensive testing has been performed with 2, 4 and 8 kHz notched-noise paradigms. All groups have mean scores within the range reported in the literature (see discussion 4.1.2.). Furthermore, frequency resolution variance was similar in control and patient groups, supporting the assumption that illness is not adversely affecting psychophysical performance. However, it should be remembered that symmetrical measurements are relatively simplistic and can be distorted by underlying asymmetric filter shapes, especially with sensorineural loss (Tyler et al., 1984). Nevertheless, inter-supporting significant differences were not apparent at any frequency; although at 4 kHz left ears had significantly poorer frequency

resolution in adult patients (p = 0.084 and 0.025, respectively excluding and including patients with bilateral sensorineural loss) compared with adult control subjects. The analysis presented here (results 3.4.4.) - of much larger groups - overturns the preliminary finding of Mulheran et al. (1997), that saw a significantly higher degree of variance in the controls when compared with patients at 2 kHz. A deficit at 4 kHz but not at the frequencies to either side (2 and 8 kHz) initially seems difficult to reconcile with an effect progressing downwards in frequency. However if 8 kHz was not as reliable a measure of frequency resolution - possibly due to inter-individual variability increasing with higher frequencies (Figures 3.4.2.1-3.) - then a mid-frequency deficit might be expected. If there is no measurable deficit, either a real loss is not being detected because the method is too variable, or there is no loss and no change in sharp tuning of the cochlea at the frequencies tested. A deficit at 4 kHz frequency resolution - but not at 2 or 8 kHz - could then be attributed to an optimisation of test sensitivity and specificity. If high frequency measurements were more variable than low frequency measurements, signs of cochleotoxicity may be detected earlier at mid-frequencies. The test to show the earliest signs of change would then be a compromise, balancing the largest with the least variable response. Alternatively, the measure of frequency resolution at 4 kHz may be the highest frequency at which selectivity was reliably assessed, as frequency resolution was not demonstrated to be independent of PTA at 8 kHz.

4.4.5. OAES

A deficit in OAEs indicates a functional loss, only up to - but not including - the level of the IHCs. Also, it should be remembered that DPOAEs reflect the functional state of OHCs at the place of their generation and not the frequency of the stimuli. In addition, an OAE deficit may be due to middle ear changes at that frequency, and would require multi-frequency tympanometry to confirm the loss is of cochlear origin. Patient measurements will also be affected to a greater or lesser

extent by: crepitations, wheezes and coughs (or the suppression of coughs). Consequently, patients can be expected to have decreased concentration and increased background noise, artificially elevating subjective thresholds as well as OAE noise floors (in extreme cases SOAEs are unobtainable*). Furthermore, although increased probe insertion depth can be expected to decrease background noise, variations in volume between ILO probe and eardrum and 'checkfit' were assumed to be equal between patient and control subjects.

4.4.6. DP-GRAMS

Overall, the mean DP-grams for control subjects and CF patients without sensorineural loss are very similar, and conform with existing work (Degg, 1995; Vinck et al., 1996). However, the patients with sensorineural loss have $2f_1$-f_2 intensities below the 2 SD limit of control values from 3-4 kHz (see Figures 3.5.4.A. and B.). These DP-gram deficits match the progression of loss shown by pure tone audiometry, and provide objective evidence for a deficit of cochlear origin that involves loss in OHC function. Thus, the mean DP-grams for the control groups will enable quick comparison for future individual patient DP-grams, with the possibility of revealing signs of hearing loss prior to pure tone audiometry (as might have been possible with the patients who now exhibit more severe losses).

4.4.7. DPOAE INPUT-OUTPUT FUNCTIONS

For DPOAE input-output functions, Ohlms et al. (1991) showed an elevated iso-criterion and increasing slope with non-ototoxic sensorineural loss in patients. One possible model to explain the relationship between iso-criterion and slope can be proposed: changes in slope could be thought to reflect changes in gain; whereas changes in

---

* Patients with the highest aminoglycoside exposure will tend to be the most ill, and therefore more noisy resulting in their exclusion (thus being underrepresented in SOAE measures).

iso-criterion could be thought to reflect changes in coupling within the cochlea. In other words, a worse iso-criterion may represent an increased phase cancellation or decreased level-dependent superposition of OHC contribution between two generator sites (see introduction 1.8.11.). On the other hand, a steeper DPOAE input-output gradient may represent a lower overall output from OHCs at that frequency. To elaborate further, slopes of 1.0 or less correlate with normal PTA thresholds (Bonfils & Avan, 1992), whereas slopes greater than 1.0 appear to occur at higher stimulus levels and with sensorineural loss (Ohlms et al., 1991). To achieve this, the origin of normal and impaired input-output functions would appear to dissociate: shallower input-output functions occurring over lower stimulus levels with normal hearing; and, steeper input-output functions occurring over higher stimulus levels with sensorineural loss. It therefore follows that for a input-output function attributed to sensorineural loss, to achieve the same level of response as normal but with an increased slope, any OHC contribution must be over a narrower stimulus range. Achieving the same DPOAE output for the less input (or greater output for the same input) with sensorineural loss may seem counter-intuitive. However as previously mentioned (discussion 4.1.2.), it is likely that at higher stimulus levels there is less active OHC contribution and more passive mechanical contribution to the DPOAE input-output function (Lonsbury-Martin et al., 1990a). Thus, an increased slope may still reflect a lower OHC contribution. Of course, these conjectures are only preliminary and further research in both humans and animals is needed to test such suppositions.

DPOAE input-output functions were found to have a wide range of 'normal' iso-criteria (20-65 dBSPL) and slopes (0.2-1.8) depending on test frequency. Furthermore, sample size was sufficient to detect any difference between patient and control groups of 5 dB or more in iso-criterion and 0.2 or more in slope. However, inter-supporting significant differences in input-output function iso-criterion and slope were not apparent, even when including patients with bilateral sensorineural loss.

Thus, a larger sample has failed to confirm the findings published by Mulheran & Degg (1996) who found a significant deficit in growth rate iso-threshold at 4 kHz, (explained then by a synergistic interaction between aminoglycosides and noise). In any case, a loss progressing downwards in frequency seems difficult to reconcile as compatible with their finding - particularly with an objective measure - of a loss at 4 kHz but not at the frequencies to either side (2 and 6 kHz).

Nevertheless, it might still be reasonable to expect a change in the proportion of different input-output function shape types between patients and control subjects. DPOAE input-output functions are known to change in shape over a narrow frequency range (He & Schmiedt, 1993), and have been shown here (results 3.6.1.) to fluctuate with time. All else being equal - if exposed and non-exposed groups are compared - it should still be possible to detect differences.* It was expected, that the proportion of shapes reflecting one or less generator site would increase compared with two or more generator sites; indicating a progressive loss of generator sites (see introduction 1.8.11.). Hence, a progression of damage may be reflected in a shift in shape from diphasic to flat-steep, and from flat-steep to linear (preserving the same slope of the linear component - at higher stimulus amplitudes - throughout these changes). Such subtle shape changes were not apparent. However, there were two interesting observations. Firstly, patients with bilateral sensorineural loss often had no measurable DPOAE (the prevalence of absent input-output functions increased with increasing frequency from 4-8 kHz). Secondly, patients had a higher proportion of saturating input-output functions - at the expense of linear input-output functions - when compared with control subjects (also increasing with frequency; Table 3.6.4.4.). Thus, with respect to increasing stimulus input, the maximum hair cell output could be being reached earlier and may represent an OHC equivalent to recruitment.

---

* It is also necessary to assume that any shift in frequency of generation site with changing stimulus intensity would have an equal effect on all groups.

4.4.8. SOAES

OHCs are thought to act as amplifiers within the cochlea (Neely & Kim, 1986). It is a possibility that OHCs use a reciprocal of the same mechanism to also act as attenuators, in order to maximise the signal-to-noise ratio in the cochlea. In other words, OHC contractility may act to damp in synchrony with the amplification process at complimentary locations along the cochlear partition in order to optimise sensitivity and selectivity. If so, it is possible to further speculate that cochlear noise might be expected to increase if damage uncoupled the attenuating damping process (i.e. OHCs may lose the ability to positively damp, prior to loosing the ability to amplify). Thus, there may initially be an increase prior to a decrease in OHC 'gain' (manifest as temporarily 'improved' OAEs; Brown et al., 1989) to compensate for damage (possibly to inner hair cells; Wake et al., 1996). However, mean 'cochlear' noise spectra for patients and control subjects were practically identical above 2.5-3 kHz (see Figures 3.7.5.1.A. and B.). Furthermore there were no significant differences ($p > 0.05$) in occurrence, frequency (Hz) nor amplitude of SOAEs between controls and patients. Nevertheless, none of the patients with sensorineural loss who were tested exhibited any SOAEs. Therefore, occurrence would appear to be potentially the most sensitive retrospective indicator of SOAE change, as absence of any SOAEs severely limits the statistical power for comparisons of frequency and amplitude.

4.4.9. CSSOAES

For CSSOAEs, sample size was sufficient to detect any difference between patient and control groups of 500 Hz or more in frequency and 3 dB or more in amplitude. Even so, there were no consistent significant differences ($p > 0.05$) in occurrence, frequency (Hz), or amplitude of CSSOAEs, even when including patients with bilateral sensorineural loss. An indication of a loss in SOAEs before CSSOAEs might be expected if SOAEs present a higher continuous functional demand upon the OHCs

than CSSOAEs, and the functional capacity of OHCs is compromised (Burr et al., 1998). Furthermore, the CSSOAE method may include a small but significant transient-evoked component that could be expected to be more resistant to detecting changes in OHC function.

4.4.10. PROGRESSION OF COCHLEAR DAMAGE

Frequency resolution may be a more relevant measure of the perceptual consequences of ototoxicity than OAEs, but appears to be a less sensitive measure. As mentioned previously (methods 2.6.3. and results 3.4.), it is anticipated that a much more thorough analysis of the frequency resolution data will be undertaken prior to further publication and once the computer program has become available. Of course it is possible that such an analysis will reveal differences indicating a change to the order of deficits as indicated here. However, it must be remembered that the relative sensitivities of different tests precludes identification of a progression of deficits in structure-specific performance. If one test shows a deficit; it is not necessarily the corresponding function that is affected first, it may be that the test is more sensitive to change (e.g. SOAEs vs. frequency resolution). As anticipated, the results indicate the progression of deficit detection by tests to be: OAEs > frequency resolution > PTA. However, to confirm the order of detection, a longitudinal study to correlate PTA changes with a progressive loss of performance in other tests is needed.

4.4.11. SAMPLE SIZE

With the exception of SOAEs, power analyses confirm sufficient group sizes to detect any biologically plausible significant differences, should they exist. Increasing the number of patients tested above the number presented here (results 3.1.3.) has implications for ensuring a representative sample. If 'new' patients were now approached from existing collaborating hospitals, these will mostly be individuals who have recently become of an age suitable for testing, hence biasing the data

towards the youngest and least exposed individuals. If existing patients are re-approached and coerced into testing,* patients who have previously refused to take part are more likely to be less co-operative and hence be less conscientious in the performance of psychophysical tests. Furthermore, any reassessment of patients must consider that some patients are now deceased and these will tend to represent those individuals with high aminoglycoside exposures.

4.4.12. RECOMMENDED PROTOCOL ALTERATIONS

Since this study commenced, evidence has been found in the literature recommending the use of 5 rather than 4 masking noise conditions for notched noise frequency resolution in order to optimise variability (Leeuw & Dreschler, 1994). In addition, the DPOAE stimulus-recording parameters would be retained with one exception. With hindsight, to optimise the amplitude of the $2f_1$-f_2 distortion product: I_1 would still be 70 dBSPL, to maximise the available range for input-output function measurements; whereas, I_2 would be changed to 65 dBSPL, to minimise swamping of the f_1 waveform (Hauser & Probst, 1991). Also, a greater initialisation effect on SOAEs during quinine dosing and recovery has been reported compared with 'normal' test-retest cases (McFadden & Pasanen, 1994). Thus, a differential initialising effect in treatment versus non-treatment groups could confound ototoxicity studies that employ OAE measurements, and should be monitored in future.

* Apart from the ethical implications of applying pressure on patients to take part.

4.5. MECHANISTIC SUMMARY

4.5.1. TECHNIQUES EMPLOYED

The non-screening tests were: high frequency pure tone audiometry (at 10, 12, 14 and 16 kHz); frequency resolution (at 2, 4 and 8 kHz); and OAE measurements (DP-gram; DP input-output functions at 2, 4, 6 & 8 kHz; SOAEs; and CSSOAEs). For each test there were results from approximately 50 individual patients, and a similar or larger number of control subjects. The inter-aural, inter-subject and intra-subject variabilities for each test type have been calculated in control subjects. The effect of age in control subjects and the effect of dose in patients were similarly investigated for all tests. Test results have also been directly compared for CF patients and control subjects. This is the largest most comprehensively tested sample of human subjects known in the field of ototoxicity research at this point in time.

The study is also vindicated by the extensive normative data-set, which alone will provide a very useful resource for the future study of other ototoxic agents and indeed other types of auditory dysfunction in humans. In particular, we are concerned here with the identification and progression of deficits in different aspects of peripheral auditory processing. Thus the aim is to characterise the development of impairment and the associated sites of damage; by employing mechanistic methods of monitoring for ototoxicity, which are clinically transferable. An unfortunate but particularly important limiting factor in this type of research is that there will undoubtedly be some sub-clinically abnormal control subjects that can not be identified.

4.5.2. PROGRESSION OF DEFICIT BY TESTS

Overall, when comparing CF patients receiving aminoglycoside antibiotics with non-CF subjects not receiving aminoglycosides, there is an increase in the proportion of saturating DP input-output functions at 4, 6 and 8 kHz, and a marginal decrease in mean frequency resolution at 4

kHz. Possible changes in frequency resolution indicate poorer cochlear selectivity. Whilst the changes in DPOAEs indicate a lower maximal output of OHCs in response to sound stimuli. Thus, there is evidence for subtle changes in the performance of the inner ear, which confirm OHCs as a primary target, in CF patients receiving ototoxic aminoglycoside therapy. The project has only been concerned with peripheral 'sensory' toxicity; although the possibility of central 'neural' toxicity must be borne in mind. Indeed, neural degeneration without hair cell loss has been demonstrated with aminoglycoside ototoxicity in humans (Hinojosa & Lerner, 1987). If such cases occurred, a deficit in high frequency PTA without deficit in OAEs could have indicated VIIIth nerve damage. Thus, possible indicators for loss in different aspects of auditory performance have been highlighted.

4.5.3. ACUTE AND CHRONIC CHANGES

DPOAE tests (being objective measurements) have been performed before, during, and after gentamicin and tobramycin intravenous infusions, on two separate individuals (Burr, not reported here). In both cases there was no apparent change in DPOAEs. The outcome provides important evidence against a short-term fluctuating / reversible deficit, which could otherwise confound interpretation of data collected at different times with respect to aminoglycoside infusions. However, animal studies suggest that aminoglycoside entry into cochlear fluids is relatively slow (requiring approximately 3 hours before plateau concentration in the rat; Tran-Ba-Huy, 1986) and so little acute effect would be expected at therapeutic levels. Nevertheless, neither of these patients had any SOAEs nor CSSOAEs that could be monitored, and both patients had been established on a regular regime of therapy for some years. Therefore, acute monitoring of SOAEs and CSSOAEs particularly in a patient unaccustomed to therapy would be more interesting (if such an individual would agree to be studied). Thus, despite a lack of convincing evidence of any substantial chronic changes with OAEs, it is

still possible that acute changes may occur. As with aspirin, DPOAEs may only be affected when associated with transient changes in SOAEs (Burr, unpublished work). Furthermore, with aminoglycoside ototoxicity there may be an increase in OAE activity prior to a loss (Brown et al., 1989), which may confound efforts of acute monitoring. Hence, it is quite possible that a transient change - occurring suddenly, or over a short period of time - is being missed by this design of study. A more thorough longitudinal study monitoring SOAEs for change in individuals with little or no prior exposure should reveal these more subtle changes should they occur.

It is possible that an individual is only predisposed to ototoxicity during the first exposure, and further deficits only occur when the body is subsequently compromised in other ways (e.g. renal failure). Therefore, acute monitoring may not reveal short-term reversible changes, but there may still be an acute reversible effect with the first ('conditioning' / loading) dose. Also, onset and degree of ototoxicity might be either dependent on dose or independent of dose. Furthermore, the time course of efficacy suggests giving more aminoglycoside less often within each course of treatment in order to minimise the growth of resistant bacteria (Begg & Barclay, 1995). However, the time course of toxicity is relatively unknown and recent indications of fewer toxicity cases (with less frequent but higher doses) appear to be based on very poorly controlled indices (e.g. Wood et al., 1996).

4.5.4. CONCLUSIONS

CF patients commonly receive 1-4 courses of aminoglycoside every year and yet appear to suffer a similar proportion of ototoxic side-effects to non-CF patients who have usually only ever received a single course. This obvious contradiction in expectation may be attributed to a 'beneficial' side-effect of the CF condition, whereby over-active chloride ion transporters possibly export aminoglycosides from the cochlea. Induction of multi-drug resistance proteins in the cochlea (Saito et al.,

1997) - by regular and repeated exposure to intravenous therapy - may also result in a protective effect. Furthermore with long term exposure, the more efficient excretion of aminoglycosides by CF (Finkelstein & Hall, 1979), coupled with the possibility of hair cell regeneration (Walsh et al., 1998) contributing to a slowly reversible change, may reduce the impact of aminoglycosides on the cochlea. However, even in non-CF patients, multiple aminoglycoside courses may simply cause no greater risk of side effects than a single course. Thus aminoglycoside ototoxicity in CF patients is less of a problem than would be anticipated. Nevertheless, the statistical power of this work would be greatly increased by re-testing the surviving patients in a few years time and by more precisely identifying the sources of variability in the measurement of dose.

Without a dose-response relationship it is not possible to confirm that any specifically toxic effects have occurred. The immense cumulative exposures to aminoglycoside received by many patients may not be causal and it is possible - however unlikely - that sensorineural losses are caused by the CF condition itself. For example, there may be an accelerated age-related loss associated with the CF condition. However, aminoglycoside ototoxicity remains the most plausible cause of high frequency sensorineural loss in CF patients. Perhaps the most prominent flaw in the project was the inability to accurately quantify antibiotic exposure. Lack of definition of internal dose was the single most limiting factor in this study, and unfortunately one which it was not possible to remedy. The principle reason for this is that the prescription and monitoring of intravenous aminoglycoside therapy is frequently described as a 'black art' and current practice is not substantially evidence-based.

The initial experimental design was principally to capture sub-clinical effects. In conclusion, patients have subtle tests of auditory performance on the poor side of normal, and the established clinical test of PTA revealed a 7.7% (control adjusted) accumulated risk of sensorineural loss indicative of ototoxicity. Further marginal deficits in

OAEs and frequency resolution are suggestive of OHC disruption with the additional possibility of selectivity being affected before sensitivity. Thus, non-invasive physiological measurements in humans support the toxicological mechanism in animal models. However, a higher proportion of subtle effects than profound effects was expected, so the lack of any substantial sub-clinical effects is surprising. It would suggest that hearing loss with intravenous aminoglycosides occurs suddenly and is probably too complex to predict (occurring due to a period of susceptibility within the individual patient). Therefore the present regime of mostly thrice daily dosing appears to successfully balance maximum tolerable side effects with maximum therapeutic efficacy. Overall, deficits in DPOAEs do not appear to precede PTA deficits but coincide with PTA deficits, thus rendering DPOAEs as an objective surrogate that is potentially more sensitive for measuring changes in function than PTA. Ideally, it would have been possible to identify a sub-clinical index of toxicity which changes in response to dose, but is otherwise low in variability.

Undoubtedly - as with all research - this project has provided more questions than answers. However, the project has provided valuable insight into the mechanisms of aminoglycoside ototoxicity. Guidance on the problems encountered will be useful for future clinical management and research. From the basis of this work, it is anticipated that a more extensive prospective multi-centre study will examine both the efficacy and safety of once versus thrice daily tobramycin for pulmonary exacerbation of CF.

REFERENCES

5.1. NPL STANDARDS

BS 4009:1975

'Specification for an artificial mastoid for the calibration of bone vibrators used in hearing aids and audiometers'.

BS 5969:1981 (formerly IEC 651:1979)

'Specification for sound level meters'.

BS 5724:1989 (formerly IEC 601-1:1988)

'Specifications for Medical electrical equipment, part 1:1989 General requirements for safety' .

BS 2497:1992 (formerly ISO 389:1991)

'Specification for standard reference zero for the calibration of pure tone air conduction audiometers'.

BSEN 61027:1993 (formerly IEC 1027:1991)

'Specification for instruments for the measurement of aural acoustic impedance / admittance'.

BSEN 60645-1:1995 (formerly IEC 645-1:1992)

'Specification for Audiometers, part 1. pure-tone audiometers'.

EN 27566:1991 (formerly BS 6950:1988, and ISO 7566:1987)

'Specification for a standard reference zero for the calibration of pure tone bone conduction audiometers'.

IEC 225:1966 (formerly BS 2475:1964)

'Specification for octave and one-third octave band-pass filters'.

IEC 303:1970

'Provisional reference coupler for the calibration of earphones used in audiometry'.

5.2. PAPERS AND BOOKS

ANDERSON S.D. (**1980**)

Some ECMR properties in relation to other signals from the auditory periphery

Hear. Res., 2:273-296.

ASHMORE J.F. (**1987**)

A fast motile response in guinea-pig OHCs: the cellular basis of the cochlear amplifier

J. Physiol., 388:323-347.

BACKUS R.M., DE-GROOT J.C.M.J., TANGE R.A. & HUIZING E.H. (**1987**)
Pathological findings in the human auditory system following long standing gentamicin ototoxicity
Arch. Otorhinolaryngol., 244:69-73.
BALLANTYNE D. (**1990**)
Handbook of audiological techniques
Butterworth-Heinemann Ltd., London, 237p.
BARZA M. & LAUERMANN M. (**1978**)
Why monitor serum levels of gentamicin?
Clin. Pharmacokinet., 3:202-215.
BASILE A.S., HUANG J.M., XIE C., WEBSTER D., BERLIN C. & SKOLNICK P. (**1996**)
N-Methyl-D-aspartate antagonists limit aminoglycoside antibiotic induced hearing loss
Nature Medicine, 2(12):1338-1343.
BEGG E.J. & BARCLAY M.L. (**1995**)
Aminoglycosides - 50 years on
Br. J. Clin. Pharmac., 39(6):597-603.
BERGMAN M., NAJENSON T, KORN C., HAREL N., ERENTHAL P. & SACHARTOV E. (**1992**)
Frequency selectivity as a potential measure of noise damage susceptibility
Brit. J. Audiol., 26:15-22.
BERRY M.M., STANDRING S.M. & BANNISTER L.H. (**1995**)
Auditory and vestibular apparatus
In *Gray's Anatomy, 38th ed.,*
Churchill Livingstone, London, pp. 1367-1397.
BONDING P. (**1979**)
Critical bandwidth in patients with a hearing loss induced by salicylates
Audiology, 18:133:144.
BONFILS P. & AVAN P. (**1992**)
Distortion-product otoacoustic emissions: values for clinical use
Arch. Otolaryngol. Head Neck Surg., 118:1069-1076.
BRASS D. & KEMP D.T. (**1993**)
Analyses of Mossbauer mechanical measurements indicate that the cochlea is mechanically active
J. Acoust. Soc. Am., 93(3):1502-1515.
BRITISH JOURNAL OF AUDIOLOGY (**1981**)
Recommended procedures for pure-tone audiometry using a manually operated instrument
Brit. J. Audiol., 15(3):213-216.
BRITISH JOURNAL OF AUDIOLOGY (**1985**)
Recommended procedure for pure-tone bone-conduction audiometry without masking using a manually operated instrument
Brit. J. Audiol., 19:281-282.

BRITISH JOURNAL OF AUDIOLOGY (**1986**)
Recommendations for masking in pure tone threshold audiometry
Brit. J. Audiol., 20:307-314.
BRITISH JOURNAL OF AUDIOLOGY (**1989**)
British Society of Audiology - recommended format for audiogram forms
Brit. J. Audiol., 23:265-266.
BRITISH JOURNAL OF AUDIOLOGY (**1992**)
Recommended procedure for tympanometry
Brit. J. Audiol., 26:255-257.
BRITISH NATIONAL FORMULARY Number 31 (**March 1996**)
Infections; Antibacterial drugs; Aminoglycosides
BMA and Royal Pharmaceutical Society of Great Britain, 5.1.4: 242-244.
BROWN A.M. & KEMP D.T. (**1983**)
Oto-acoustic emissions: the iso-suppression tuning properties of the distortion product $2f_1$-f_2 in gerbil and man
Br. J. Audiol., 17:123-124.
BROWN A.M. & KEMP D.T. (**1984**)
Suppressibility of the $2f_1$ - f_2 stimulated acoustic emissions in gerbil and man
Hear. Res., 13:29-37.
BROWN A.M., MCDOWEL B. & FORGE A. (**1989**)
Acoustic distortion products can be used to monitor the effects of chronic gentamicin treatment
Hear. Res., 42:143-156.
BROWNELL W.E., BADER C.R., BERTRAND D. & DE-RIBAUPIERRE Y. (**1985**)
Evoked mechanical responses of isolated cochlear outer hair cells
Sci., 227:194-196.
BRUMMETT R.E. (**1980**)
Drug-induced ototoxicity
Drugs, 19:412-428.
BRUMMETT R.E. & FOX K.E. (**1982**)
Studies of aminoglycoside ototoxicity in animal models
In *The aminoglycosides: microbiology, clinical use, and toxicology* (eds. A. Whelton & H.C. Neu)
Marcel Dekker, New York, pp. 419-451.
BURR S.A., MULHERAN M. & DEGG C. (**1997**)
Characterisation of 'Click-Synchronised Spontaneous Oto-Acoustic Emissions' (CSSOAEs) in humans
Br. J. Audiol., 31(2):93-94.
BURR S.A., MULHERAN M. & DEGG C. (**1997**)
Occurrence of spontaneous oto-acoustic emissions in a patient group receiving frequent gentamicin therapy compared with control subjects
Hum. Exp. Toxicol., 16(7):388.

BURR S.A., MULHERAN M., DEGG C. & COLLINSON J. (**1998**)
Differences between two types of spontaneous oto-acoustic emission in a patient group receiving frequent gentamicin therapy compared with control subjects
Br. J. Audiol., 32 (2):84-85.
CARNEY A.S. & BIRCHALL J.P. (**1995**)
How to use an otoscope
Student B.M.J., 3:231-233.
CHRISTOVICH L.A. (**1957**)
Frequency characteristics of the masking effect
Biofizika, 2(6):743-755.
CIPOLLI M., CANCIANI M., CAVAZZANI M., URAS P., ZAMPIERI P. & MASTELLA G. (**1993**)
Ear disease is not a common complication in cystic fibrosis
Eur. J. Pediatr., 152:265-266.
CODY A.R. & RUSSELL I.J. (**1987**)
The responses of hair cells in the basal turn of the guinea-pig cochlea to tones
J. Physiol., 383:551-569.
COLLET L., KEMP D.T., VEUILLET E., DUCLAUX R., MOULIN A. & MORGAN A. (**1990**)
Effect of contralateral auditory stimuli on active cochlear micro mechanical properties in human subjects
Hear. Res., 43:251-262.
COREY D.P. & HUDSPETH A.J. (**1979a**)
Ionic basis of the receptor potential in a vertebrate hair cell
Nature, 281:675-677.
COREY D.P. & HUDSPETH A.J. (**1979b**)
Response latency of vertebrate hair cells
Biophys. J., 26:499-506.
CRAN S.A. & SCHACHT J. (**1996**)
Activation of aminoglycoside antibiotics to cytotoxins
Audiol. Neurootol., 1:80-85.
CRIFO S., ANTONELLI M., GAGLIARDI M., LUCARELLI N. & MARCOLINI P. (**1980**)
Ototoxicity of aminoglycoside antibiotics in long-term treatment for cystic fibrosis
Int. J. Pediatr. Otorhinolaryngol., 2:251-253.
DADSON R.S. & KING J.H. (**1952**)
A determination of the normal threshold of hearing and its relation to the standardization of audiometers
J. Laryngol. Otol., 66:366-378.
DALLOS P. (**1992**)
The active cochlea
J. Neurosci., 12(12):4575-4585.

DAVEY P.G., JABEEN F.J., HARPUR E.S., SHENOI P.M. & GEDDES A.M. (**1982**)
The use of pure-tone audiometry in the assessment of gentamicin auditory toxicity
Brit. J. Audiol., 16:151-154.

DAVIS H. (**1958**)
Transmission and transduction in the cochlea
Laryngoscope, 68:359-388.

DAVIS H., BENSON R.W., COVELL W.P., FERNANDEZ C., GOLDSTEIN R., KATSUKI Y., LEGOUIX J.P., MCAULIFFE D.R. & TASAKI I. (**1953**)
Acoustic trauma in the guinea pig
J. Acoust. Soc. Am., 25(6):1180-1189.

DAYAL V.S., WHITEHEAD G.L. & SMITH E.L. (**1975**)
Gentamicin - progressive cochlear toxicity
Can. J. Otolaryngol., 4(2):348-351.

DEGG C. (**1995**)
Distortion product otoacoustic emissions (DPOE).
Results obtained from a group of otologically normal teenage subjects and a number of cystic fibrotic patients with a history of exposure to the ototoxic antibiotic, gentamicin. ***Diploma in technical audiology thesis.*** Institute of Laryngology and Otology, London, 54p.

DEGG C. & MULHERAN M. (**1996**)
The effect of frequent exposure to gentamicin on distortion product OAEs in patients with cystic fibrosis
Brit. J. Audiol., 30(2):99-100.

DE-GROOT R. & SMITH A.L. (**1987**)
Antibiotic pharmacokinetics in cystic fibrosis: differences and clinical significance
Clin. Pharmacokinet., 13:228-253.

DELGUTTE B. (**1988**)
Physiological mechanisms of masking
In *Proceedings of the 8th international symposium on hearing: Basic Issues in Hearing* (eds. H. Duifhuis, J.W. Horst & H.P. Wit) Academic Press, London, pp. 204-214.

DUIFHUIS H. (**1971**)
Audibility of high harmonics in a periodic pulse II. Time effect
J. Acoust. Soc. Am., 49(4):1155-1162.

EDSON R.S. & TERRELL C.L. (**1987**)
The aminoglycosides streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and sisomicin
Mayo. Clin. Proc., 62:916-920.

ESTERHAI J.L., BEDNAR J & KIMMELMAN C.P. (**1986**)
Gentamicin-induced ototoxicity complicating treatment of chronic osteomyelitis
Clin. Orthopaed. Res., 209:185-188.

EVANS E.F. (**1972**)
The frequency response and other properties of single fibres in the guinea-pig cochlear nerve
J. Physiol., 226:263-287.
EVANS & WILSON (**1975**)
Cochlear tuning properties: concurrent basilar membrane and single nerve fibre measurements
Sci., 190:1218-1221.
FAUSTI S.A., RAPPAPORT B.Z., SCHECHTER M.A., FREY R.H., WARD T.T. & BRUMMETT R.E. (**1984**)
Detection of aminoglycoside ototoxicity by high-frequency auditory evaluation: selected case studies
Am. J. Otolaryngol., 5:177-182.
FAUSTI S.A., HENRY J.A., SCHAFFER H.I., OLSON D.J., FREY R.H. & MCDONALD W.J. (**1992**)
High-frequency audiometric monitoring for early detection of aminoglycoside ototoxicity
J. Infect. Dis., 165:1026-1031.
FEE (**1980**)
Aminoglycoside ototoxicity in the human
Laryngoscope, 90:1-19.
FINKELSTEIN E. & HALL K. (**1979**)
Aminoglycoside clearance in patients with cystic fibrosis
J. Pediatr., 94(1):163-164.
FLETCHER H. (**1940**)
Auditory patterns
Rev. Mod. Phys., 12:47-65.
FLOTTORP G. (**1995**)
Improving audiometric thresholds by changing the headphone position at the ear
Audiology, 34:221-231.
FORMAN-FRANCO B., ABRAMSON A.L., GORVOY J.D. & STEIN T. (**1979**)
Cystic fibrosis and hearing loss
Arch. Otolaryng., 105:338-342.
FOWLER E.P. (**1936**)
A method for the early detection of otosclerosis
Arch. Otolaryng., 24:731-741.
GARETZ S., ALTSCHULER R.A. & SCHACHT J. (**1994**)
Attenuation of gentamicin ototoxicity by glutathione in the guinea pig in vivo
Hear. Res., 77(1-2):81-87.
GARETZ S., RHEE D.J. & SCHACHT J. (**1993**)
Attenuation of gentamicin ototoxicity by glutathione
Abs. Assoc. Res. Otolaryngol., 16:141.

GASKILL S.A. & BROWN A.M. (**1990**)
The behaviour of the acoustic distortion product, 2f1-f2, from the human ear and its relation to auditory sensitivity
J. Acoust. Soc. Am., 88(2):821-839.
GLANVILLE J.D., COLES R.R.A. & SULLIVAN B.M. (**1971**)
A family with high-tonal objective tinnitus
J. Laryngol. Otol., 85:1-10.
GLASBERG, B.R. & MOORE, B.C.J. (**1990**)
Derivation of auditory filter shapes from notched-noise data
Hear. Res., 47:103-138.
GOBSCH H. & TIETZE G. (**1993**)
Interrelation of spontaneous and evoked otoacoustic emissions
Hear. Res., 69:176-181.
GOLD T. (**1948**)
Hearing II: the physical basis of the action of the cochlea
Proc. R. Soc. Lond. (Ser. B), 135:492-498.
GREEN D.M. (**1988**)
Profile analysis: Auditory intensity discrimination
Oxford University Press, New York, 138p.
GREENWOOD D. (**1992**)
Antimicrobial agents
In *Medical microbiology. A guide to microbial infections: pathogenesis, immunity, laboratory diagnosis and control, 14th ed.* (eds. D. Greenwood, R.C.B. Slack & J.F. Peutherer)
Churchill Livingstone, London, pp. 67-77.
GREENWOOD D.D. (**1961**)
Auditory masking and the critical band
J. Acoust. Soc. Am., 33(4):484-502.
GREENWOOD D.D. (**1971**)
Aural combination tones and auditory masking
J. Acoust. Soc. Am., 50(2):502-543.
GRIFFIN J.P. (**1988**)
Drug-induced ototoxicity
Brit. J. Audiol., 22:195-210.
HALL J.W., HAGGARD M.P. & FERNANDES M.A. (**1984**)
Detection in noise by spectro-temporal pattern analysis
J. Acoust. Soc. Am., 76(1):50-56.
HANDWERKER H.O. (**1989**)
General sensory physiology
In *Human physiology, 2nd ed.* (eds. R.F. Schmidt & G. Thews)
Springer-Verlag, Berlin, pp. 176-195.
HARPUR E.S. (**1982**)
The pharmacology of ototoxic drugs
Brit. J. Audiol., 16:81-93.

HARRIS F.P., LONSBURY-MARTIN B.L., STAGNER B.B., COATS A.C. & MARTIN G.K. (**1989**)
Acoustic distortion products in humans: systematic changes in amplitude as a function of f_2 / f_1 ratio
J. Acoust. Soc. Am., 85(1):220-229.

HAUSER R. & PROBST R. (**1991**)
The influence of systematic primary-tone level variation L_2 - L_1 on the acoustic distortion product emission $2f_1$ - f_2 in normal human ears
J. Acoust. Soc. Am., 89(1):280-286.

HAWKINS J.E., MARQUES D.M. & CLARK C.S. (**1975**)
Noise and kanamycin interaction in the guinea pig cochlea
J. Acoust. Soc. Am., 58(1):S88-89.

HE N.J. & SCHMIEDT R.A. (**1993**)
Fine structure of the $2f_1$ - f_2 acoustic distortion product: changes with primary level
J. Acoust. Soc. Am., 94(5):2659-2669.

HEIL H., BENNANI H., ERRE J., AUROUSSEAU C. & ARAN J. (**1992**)
Kinetics of gentamicin in cochlear hair cells after chronic treatment
Acta Otolaryngol. (Stockh.), 112:272-277.

HEIL H., ERRE J., AUROUSSEAU C., BOUALI R., DULON D. & ARAN J. (**1993**)
Gentamicin uptake by cochlear hair cells precedes hearing impairment during chronic treatment
Audiology, 32:78-87.

HENLEY C.M. & SCHACHT J. (**1988**)
Pharmacokinetics of aminoglycoside antibiotics in blood, inner-ear fluids and tissues and their relationship to ototoxicity
Audiology, 27:137-146.

HINOJOSA R. & LERNER S.A. (**1987**)
Cochlear neural degeneration without hair cell loss in two patients with aminoglycoside ototoxicity
J. Infect. Dis., 156(3):449-455.

HODSON M.E. & GEDDES D.M. (**1995**)
Cystic fibrosis
Chapman and Hall, London, 439p.

HOLTZ M.A., HARRIS F.P. & PROBST R. (**1994**)
Otoacoustic emissions: an approach for monitoring aminoglycoside-induced ototoxicity
Laryngoscope, 104(9):1130-1134.

HOOD J.D. (**1972**)
Fundamentals of identification of sensori-neural hearing loss
Sound, 6:21-26.

HOUTGAST T. (**1972**)
Psychophysical evidence for lateral inhibition in hearing
J. Acoust. Soc. Am., 51(6):1885-1894.

HOUTGAST T. (**1977**)
Auditory-filter characteristics derived from direct-masking data and pulsation threshold data with a rippled-noise masker
J. Acoust. Soc. Am., 62(2):409-415.
HUANG M.Y. & SCHACHT J. (**1990**)
Formation of a cytotoxic metabolite from gentamicin by liver
Biochem. Pharmacol., 40(11):R11-R14.
HUDSPETH A.J. (**1985**)
The cellular basis of hearing: the biophysics of hair cells
Sci., 230:745-752.
JACKSON G.G. & ARCIERI G. (**1971**)
Ototoxicity of gentamicin in man: a survey and controlled analysis of clinical experience in the united states
J. Infect. Dis., 124(Suppl.):130-137.
JERGER J. & JERGER S. (**1975**)
A simplified tone decay test
Arch. Otolaryng., 101:403-407.
JOHNSON-DAVIS D. & PATTERSON R.D. (**1979**)
Psychophysical tuning curves: restricting the listening band to the signal region
J. Acoust. Soc. Am., 65(3):765-770.
JOHNSSON L.G., HAWKINS J.E., KINGSLEY T.C., BLACK F.O. & MATZ G.J. (**1980**)
Aminoglycoside-induced cochlear pathology in man
Acta Otolaryng., 383(Suppl.):3-19.
JOHNSTONE B.M. & SELLICK P.M. (**1972**)
The peripheral auditory apparatus
Quart. Revs. Biophys., 5:1-57.
JOHNSTONE B.M., PATUZZI R. & YATES G.K. (**1986**)
Basilar membrane measurements and the travelling wave
Hear. Res., 22:147-153.
KARLSSON K.K., BERNINGER E. & ALVAN G. (**1991**)
The effect of quinine on psychoacoustic tuning curves, stapedius reflexes and evoked otoacoustic emissions in healthy volunteers
Scand. Audiol., 20:83-90.
KAHLMETER G. & DAHLAGER J.I. (**1984**)
Aminoglycoside toxicity - a review of clinical studies published between 1975 and 1982
J. Antimicrob. Chemother., 13(Suppl. A):9-22.
KAYE D., LEVISON M.E. & LABOVITZ E.D. (**1974**)
The unpredictability of serum concentrations of gentamicin: Pharmacokinetics of gentamicin in patients with normal and abnormal renal function
J. Infect. Dis., 130(2):150-154.
KEENE M. & GRAHAM J.M. (**1984**)
Clinical monitoring of the effects of gentamicin by electrocochleography
J. Laryngol. Otol., 98:11-21.

KELLY D.R., NILO E.R. & BERGGREN R.B. (**1969**)
Deafness after topical neomycin wound irrigation
N. Engl. J. Med., 280(24):1338-1339.
KEMP D.T. (**1978**)
Stimulated acoustic emissions from within the human auditory system
J. Acoust. Soc. Am., 64(5):1386-1391.
KEMP D.T. (**1979**)
Evidence of mechanical nonlinearity and frequency selective wave amplification in the cochlea
Arch. Otorhinolaryng., 224:37-45.
KEMP D.T. (**1981**)
Physiologically active cochlear micromechanics - one source of tinnitus
Ciba Found. Symp., 85:54-81.
KEMP D.T., BRAY P., ALEXANDER L. & BROWN A.M. (**1986**)
Acoustic emission cochleography - practical aspects
Scand. Audiol. Suppl. 25, 15:71-95.
KEMP D.T., RYAN S. & BRAY P. (**1990**)
A guide to the effective use of otoacoustic emissions
Ear. Hear., 11(2):93-105.
KIM D.O., MOLNAR C.E. & MATTHEWS J.W. (**1980**)
Cochlear mechanics: nonlinear behaviour in two-tone responses as reflected in cochlear-nerve-fiber responses and in ear-canal sound pressure
J. Acoust. Soc. Am., 67(5):1704-1721.
KUIJPERS W. & BONTING S.L. (**1969**)
Studies on the (Na^+-K^+)-activated ATPase. XXIV. Localization and properties of ATPase in the inner ear of the guinea pig
Biochem. Biophys. Acta., 173:477-485.
LAST P.M. & SHERLOCK S. (**1960**)
Systemic absorption of orally administered neomycin in liver disease
N. Engl. J. Med., 262(8):385-389.
LAUTERMANN J., MCLAREN J. & SCHACHT J. (**1995**)
Glutathione protection against gentamicin ototoxicity depends on nutritional status
Hear. Res., 86(1-2):15-24.
LEEUW A. & DRESCHLER W.A. (**1994**)
Frequency-resolution measurements with notched noises for clinical purposes
Ear. Hear., 15:240-255.
LERNER S.A., SELIGSOHN R., BHATTACHARYA I., HINOJOSA R. & MATZ G.J. (**1982**)
Aminoglycoside levels in the inner ear perilymph of man
Inserm., 102:387-405.

LEVITT H. (**1971**)
Transformed up-down methods in psychoacoustics
J. Acoust Soc. Am., 49(2):467-477.
LI S.C., BOWES G., IOANNIDES-DEMOS L.L., SPICER W.J., HOOPER R.E., SPELMAN D.W., TONG N. & MCLEAN A.J. (**1991**)
Dosage adjustment and clinical outcomes of long-term use of high dose tobramycin in adult cystic fibrosis patients
J. Antimicrob. Chemother., 28(Suppl. A):561-568.
LIBERMAN M.C. & DODDS L.W. (**1984**)
Single neuron labelling and chronic cochlear pathology. III. Stereocilia damage and alterations of threshold tuning curves
Hear. Res., 16:55-74.
LONG G.R., TUBIS A. & JONES K.L. (**1991**)
Modeling synchronization and suppression of spontaneous otoacoustic emissions using Van der Pol oscillators: effects of aspirin administration
J. Acoust. Soc. Am., 89(3):1201-1212.
LONSBURY-MARTIN B.L., CUTLER W.M. & MARTIN G.K. (**1991**)
Evidence for the influence of ageing on distortion-product otoacoustic emissions
J. Acoust. Soc. Am., 89(4):1749-1759.
LONSBURY-MARTIN B.L., HARRIS F.P., STAGNER B.B., HAWKINS M.D. & MARTIN G.K. (**1990a**)
Distortion product emissions in humans: I. basic properties in normally hearing subjects
Ann. Otol. Rhinol. Laryngol., 99(5, Suppl. 147):3-14.
LONSBURY-MARTIN B.L., HARRIS F.P., STAGNER B.B., HAWKINS M.D. & MARTIN G.K. (**1990b**)
Distortion product emissions in humans: II. relations to acoustic immitance and stimulus frequency and spontaneous otoacoustic emissions in normally hearing subjects
Ann. Otol. Rhinol. Laryngol., 99(5, Suppl. 147):15-29.
LONSBURY-MARTIN B.L., PROBST R., SCHEININ S.A. & COATS A.C. (**1987**)
Acoustic distortion products in rabbit ear canal: II. Sites of origin revealed by suppression contours and pure-tone exposures
Hear. Res., 28:191-208.
LUNDQUIST P. & WERSALL J. (**1967**)
The ototoxic effect of gentamicin - an electron microscopical study
In *Gentamicin: First International Symposium*
Essex, Chemie Ag, Lucerne, Paris, pp. 26-46.
MARGOLIS R.H. & TRINE M.B. (**1997**)
Influence of middle-ear disease on otoacoustic emissions
In *Otoacoustic emissions: Clinical applications* (eds. Robinette M.S. & Glattke T.J.)
Thieme, New York, pp. 130-150.

MARTIN G.K., LONSBURY-MARTIN B.L., PROBST R., SCHEININ S.A. COATS A.C. (**1987**)
Acoustic distortion products in rabbit ear canal: II. Sites of origin revealed by suppression contours and pure-tone exposures
Hear. Res., 28:191-208.

MARTIN G.K., OHLMS L.A., FRANKLIN D.J., HARRIS F.P. & LONSBURY MARTIN B.L. (**1990**)
Distortion product emissions in humans: III. Influence of sensorineural hearing loss
Ann. Otol. Rhinol. Laryngol., 99(5, Suppl. 147):30-42.

MATZ G.J. & LERNER S.A. (**1981**)
Prospective studies of aminoglycoside ototoxicity in adults.
In *Aminoglycoside ototoxicity* (eds. Lerner S.A., Matz G.J. & Hawkins J.E.)
Little Brown and Company, Boston, pp. 327-336.

MAYER A.M. (**1894**)
Researches in acoustics - No. IX
Lond. Edinb. Dubl. Phil. Mag., 37(S5):259-288.

MELICHAR I. & SYKA J. (**1987**)
Electrophysiological measurements of the stria vascularis potentials in vivo
Hear. Res., 25:35-43.

MEYERS R.M. (**1970**)
Ototoxic effects of gentamicin
Arch. Otolaryng., 92:160-162.

MCFADDEN D. & PASANEN E.G. (**1994**)
Otoacoustic emissions and quinine sulfate
J. Acoust. Soc. Am., 95(6):3460-3474.

MCFADDEN D. & PLATTSMIER H.S. (**1984**)
Aspirin abolishes spontaneous oto-acoustic emissions
J. Acoust. Soc. Am., 76(2):443-448.

MILLER J.J. (**1985**)
Handbook of ototoxicity
CRC Press Inc., Florida, 321p.

MORGAN D.W., PEARMAN K., SHENOI P.M. & STABLEFORTH D. (**1988**)
Ototoxicity in adult cystic fibrosis: relationship to aminoglycoside blood levels, total dose, and duration of treatment
Thorax, 43:236P.

MOORE B.C.J. (**1989**)
An introduction to the psychology of hearing, 3rd ed.,
Academic Press Limited, London, 350p.

MOORE B.C.J. (**1995**)
Perceptual consequences of cochlea damage
Oxford University Press, Oxford, 232p.

MOORE B.C.J. & GLASBERG B.R. (**1983**)
Suggested formulae for calculating auditory-filter bandwidths and excitation patterns
J. Acoust. Soc. Am., 74(3):750-753.

MOORE B.C.J., LAURENCE R.F. & WRIGHT D. (**1985**)
Improvements in speech intelligibility in quiet and in noise produced by two channel compression hearing aids
Br. J. Audiol., 19:175-187.
MOULIN A., COLLET L., DELLI D. & MORGAN A. (**1991**)
Spontaneous otoacoustic emissions and sensori-neural hearing loss
Acta Otolaryngol. (Stockh.), 111:835-841.
MOUNTAIN D.C. (**1980**)
Changes in endolymphatic potential and crossed olivocochlear bundle stimulation alter cochlear mechanics
Sci., 210:71-72.
MULHERAN M., DEGG C., COLLINSON J. & FLETCHER S. (**1996**)
The effects of gentamicin on auditory function in humans
Hum. Exp. Toxicol., 15(2):146.
MULHERAN M. & DEGG. (**1997**)
Comparison of distortion product OAE generation between a patient group requiring frequent gentamicin therapy and control subjects
Br. J. Audiol., 31(1):5-9.
MULHERAN M., BURR S.A. & DEGG C. (**1997**)
Frequency resolution in audiologically normal young subjects compared with a patient group receiving frequent gentamicin therapy
Br. J. Audiol., 31(2):93.
MULHERIN D. & FITZGERALD M. (**1992**)
Cystic fibrosis in adolescents and adults. The coming of age of cystic fibrosis
Dig. Dis., 10(1):29-37.
MULHERIN D., FAHY J., GRANT W., KEOGAN M., KAVANAGH B. & FITZGERALD M. (**1991**)
Aminoglycoside induced ototoxicity in patients with cystic fibrosis
Ir. J. Med. Sci., 160(6):173-175.
NEELY S.T. & KIM D.O. (**1986**)
A model for active elements in cochlear biomechanics
J. Acoust. Soc. Am., 79(5):1472-1480.
NELSON D.A. & KIMBERLEY B.P. (**1992**)
Distortion product emissions and auditory sensitivity in human ears with cochlear hearing loss
J. Speech Hear. Res., 35:1142-1159.
NORTHERN J.L. & RATKIEWICZ B. (**1985**)
The quest for high frequency normative data
Semin. Hear., 6(4):331-339.
O'DONOGHUE G.M., BATES G.J. & NARULA A.A. (**1992**)
Clinical ENT: An Illustrated Textbook
Oxford University Press, Oxford, 223p.

OFFNER F.F., DALLOS P. & CHEATHAM M.A. (**1987**)
Positive endocochlear potential: mechanism of production by marginal cells of stria vascularis
Hear. Res., 29:117-124.
OHLMS L.A., LONSBURY-MARTIN B.L. & MARTIN G.K. (**1991**)
Acoustic-distortion products: separation of sensory from neural dysfunction in sensorineural hearing loss in human beings and rabbits
Otolaryngol. Head Neck Surg., 104:159-174.
PATTERSON R.D. (**1976**)
Auditory filter shapes derived with noise stimuli
J. Acoust. Soc. Am., 59(3):640-654.
PATTERSON R.D. & MOORE B.C.J. (**1986**)
Auditory filters and excitation patterns as representations of frequency resolution
In *Frequency Selectivity in Hearing* (ed. B.C.J. Moore)
Academic Press, London, pp. 123-177.
PATTERSON R.D. & NIMMO-SMITH I. (**1980**)
Off-frequency listening and auditory-filter asymmetry
J. Acoust. Soc. Am., 67(1):229-245.
PATTERSON R.D., NIMMO-SMITH I., WEBER D.L. & MILROY R. (**1982**)
The deterioration of hearing with age: frequency selectivity, the critical ratio, the audiogram, and speech threshold
J. Acoust. Soc. Am., 72(6):1788-1803.
PATUZZI R. (**1993**)
Otoacoustic emissions and the categorization of cochlear and retro-cochlear lesions
Brit. J. Audiol., 27:91-95.
PEDERSEN S.S., JENSEN T., OSTERHAMMEL D. & OSTERHAMMEL P. (**1987**)
Cumulative and acute toxicity of repeated high-dose tobramycin treatment in cystic fibrosis
Antimicrob. Agents Chemother., 31(4):594-599.
PENNER M.J., GLOTZBACH L. & HUANG T. (**1993**)
Spontaneous otoacoustic emissions: measurement and data
Hear. Res., 68:229-237.
PICKLES J.O. (**1986**)
The neurophysiological basis of frequency selectivity
In *Frequency Selectivity in Hearing* (ed. B.C.J. Moore)
Academic Press, London, pp. 51-121.
PICKLES J.O. (**1987**)
The physiology of the ear
In *Scott-Brown's Otolaryngology 5th ed. Vol. 1. Basic Sciences* (ed. D. Wright)
Butterworths, London, pp. 47-80.
PICKLES J.O. (**1988**)
An introduction to the physiology of hearing, 2nd ed.,
Academic Press Limited, London, 367p.

PICKLES J.O., COMIS S.D. & OSBORNE M.P. (**1984**)
Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction
Hear. Res., 15:103-112.
PRATT S.R. & COMIS S.D. (**1982**)
Chronic effects of loop diuretics on the guinea-pig cochlea
Brit. J. Audiol., 16:117-122.
PRIEVE B.A. & FALTER S.R. (**1995**)
COAEs and SSOAEs in adults with increased age
Ear Hear., 16:521-528.
PROBST R. & BECK D. (**1987**)
Influence of general anaesthesia on spontaneous otoacoustic emissions
Assoc. Res. Otolaryngol. Abstr., 10:17.
PROBST R., LONSBURY-MARTIN B.L. & MARTIN G.K. (**1991**)
A review of otoacoustic emissions
J. Acoust. Soc. Am., 89(5):2027-2067.
PUEL J.L., LENOIR M. & UZIEL A. (**1987**)
Dose-dependent changes in the rat cochlea following aminoglycoside intoxication. I. Physiological study
Hear. Res., 26:191-197.
PUEL J.L., DURRIEU J.P., REBILLARD G., VIDAL D., ASSIE R. & UZIEL A. (**1995**)
Comparison between auditory brainstem responses and distortion products otoacoustic emissions after temporary threshold shift in guinea pig
Acta Acustica, 3:75-82.
QUICK C.A. (**1980**)
Chapter 36. Chemical and drug effects on the inner ear
In *Otolaryngology, 2nd. ed., Volume II: The ear*
W.B. Saunders Company, Philadelphia, pp. 1804-1827.
RAMSDEN R.T. (**1981**)
Transtympanic electrocochleographic monitoring of the immediate effects of tobramycin and gentamicin
In *Aminoglycoside ototoxicity* (eds. Lerner S.A., Matz G.J. & Hawkins J.E.)
Little Brown and Company, Boston, pp. 255-268.
RHODE (**1978**)
Some observations on cochlear mechanics
J. Acoust. Soc. Am., 64:158-176.
RIESZ R.R. (**1928**)
Differential intensity sensitivity of the ear for pure tones
Physical Review, 31:867-875.
ROBINETTE M.S. & GLATTKE T.J. (**1997**)
Otoacoustic emissions: Clinical applications
Thieme, New York, 356p.

ROSSI G. & SOLERO P. (**1988**)
Evoked otoacoustic emissions (EOAE) and bone conduction stimulation
Acta Otolaryngol. (Stockh.), 105:591-594.
RUGGERO M.A., ROBLES L., RICH N.C. & COSTALUPES J.A. (**1986**)
Basilar membrane motion and spike initiation in the cochlear nerve
In *Auditory Frequency Selectivity* (ed. B.C.J. Moore & R.D. Patterson)
Plenum Press, New York, pp. 189-198.
RUSSELL I.J. & SELLICK P.M. (**1978**)
Intracellular studies of hairs cells in the mammalian cochlea
J. Physiol., 284:261-290.
SAITO T., ZHANG Z.J., TSUZUKI H., OHTSUBO T., YAMADA T., YAMAMOTO T. & SAITO H. (**1997**)
Expression of P-glycoprotein in inner ear capillary endothelial cells of the guinea pig with special reference to blood-inner ear barrier
Brain Res., 767(2):388-392.
SCHACHT J. (**1986**)
Molecular mechanisms of drug-induced hearing loss
Hear. Res., 22:297-304.
SCHACHT, J. (**1993**)
Biochemical basis of aminoglycoside ototoxicity
Otolaryngol. Clin. North Am., 26(5):845-856.
SCHACHT J. & ZENNER H.P. (**1986**)
The phosphoinositide cascade in isolated hair cells: possible role as second messenger for motile responses
Hear. Res., 22:94.
SCOTT P.M.J. & GRIFFITHS M.V. (**1994**)
A clinical review of ototoxicity
Clin. Otolaryngol., 19:3-8.
SELLICK P.M., PATUZZI R. & JOHNSTONE B.M. (**1982**)
Measurement of basilar membrane motion in the guinea pig using the Mossbauer technique
J. Acoust. Soc. Am., 72(1):131-141.
SHAILER M.J., MOORE B.C.J., GLASBERG B.R. & WATSON N. (**1990**)
Auditory filter shapes at 8 and 10 kHz
J. Acoust. Soc. Am., 88(1):141-148.
SIBER G.R., ECHEVERRIA P., SMITH A.L., PAISLEY J.W. & SMITH D.H. (**1975**)
Pharmacokinetics of gentamicin in children and adults
J. Infect. Dis., 132(6):637-651.
SIVIAN L.J. & WHITE S.D. (**1933**)
On minimum audible sound fields
J. Acoust. Soc. Am., 4:288.
SMITH C.R., BAUGHMAN K.L., EDWARDS C.Q., ROGERS J.F. & LIETMAN P.S. (**1977**)
Controlled comparison of amikacin and gentamicin
N. Engl. J. Med., 296:349-353.

SMURZYNSKI J. & PROBST R. (**1996**)
Error in the calculation of synchronized spontaneous otoacoustic emission frequencies measured with the ILO88 system
J. Acoust. Soc. Am., 100(4):2555-2557.
SMURZYNSKI J. & PROBST R. (**1998**)
The influence of disappearing and reappearing spontaneous otoacoustic emissions on one subject's threshold microstructure
Hear. Res., 115:197-205.
SPOENDLIN H. (**1970**)
Structural basis of peripheral frequency analysis
In *The proceedings of the international symposium on frequency analysis and periodicity detection in hearing* (eds. R. Plomp & G.F. Smoorenberg)
Sijthoff, Leiden, pp. 2-40.
SPOENDLIN H. (**1972**)
Innervation densities of the cochlea
Acta Otolaryng., 73:235-248.
SPOENDLIN H. (**1978**)
The afferent innervation of the cochlea
In *Evoked Electrical Activity in the Auditory Nervous System* (eds. R.F. Naunton & C. Fernandez)
Academic Press, London, pp. 21-41.
SPOENDLIN H. (**1984**)
Efferent innervation of the cochlea
In *Comparative Physiology of Sensory Systems* (eds. L. Bolis, R.D. Keynes & S.H.P. Maddrell)
Cambridge University Press, Cambridge, pp. 163-188.
STEPHEN R.O. & BADHAM N.J. (**1996**)
The effects on transient evoked otoacoustic emissions following changes in external auditory canal acoustic impedance
Audiology, 35:180-193.
STONE M.A., GLASBERG B.R. & MOORE B.C.J. (**1992**)
Simplified measurement of auditory filter shapes using the notched-noise method
Br. J. Audiol., 26:329-334.
SUN X., KIM D.O., JUNG M.D. & RANDOLPH K.J. (**1995**)
The performance of distortion product otoacoustic emission test of sensorineural hearing loss in humans: comparison of stimuli with L1 > L2 and L1 = L2
Assoc. Res. Otolaryngol. Meeting Abstr., 18:125.
TAKADA A. & SCHACHT J. (**1982**)
Calcium antagonism and reversibility of gentamicin-induced loss of cochlear microphonics in the guinea pig
Hear. Res., 8:179-186.

TANGE R.A., DRESCHLER W.A., PRINS J.M., BULLER H.R., KUIJPER E.J. & SPEELMAN P. (**1995**)
Ototoxicity and nephrotoxicity of gentamicin vs netilmicin in patients with serious infections. A randomized clinical trial
Clin. Otolaryngol., 20:118-123.

TANGE R.A., DRESCHLER W.A. & VAN-DER-HULST R.J.A.M. (**1985**)
The importance of high tone audiometry in monitoring of ototoxicity
Arch. Otorhinolaryngol., 242:77-81.

TASAKI I. (**1954**)
Nerve impulses in individual auditory nerve fibres of guinea pig
J. Neurophysiol., 17:97-122.

TASAKI I. & SPYROPOULOS C.S. (**1959**)
Stria vascularis as a source of endocochlear potential
J. Neurophysiol., 22:149-155.

TRAN-BA-HUY P., BERNARD P. & SCHACHT J. (**1986**)
Kinetics of gentamicin uptake and release in the rat: comparison of inner ear tissues and fluids with other organs
J. Clin. Invest., 77:1492-1500.

TYLER R.S., HALL J.W., GLASBERG B.R., MOORE B.C.J. & PATTERSON R.D. (**1984**)
Auditory filter asymmetry in the hearing impaired
J. Acoust. Soc. Am., 76(5):1363-1368.

ULEHLOVA L., VOLDRICH L. & JANISCH R. (**1987**)
Correlative study of sensory cell density and cochlear length in humans
Hear. Res., 28:149-151.

VINCK B.M., VEL E.D., XU Z.M. & CAUWENBERGE P.B.V. (**1996**)
Distortion product otoacoustic emissions: a normative study
Audiology, 35(5):231-245.

VON-BEKESEY G. (**1947**)
The variation of phase along the basilar membrane with sinusoidal vibrations
J. Acoust. Soc. Am., 19(3):452-460.

VON-BEKESEY G. (**1952**)
DC resting potentials inside the cochlear partition
J. Acoust. Soc. Am., 24(1):72-76.

WABLE J.W. & COLLET L (**1994**)
Can synchronised otoacoustic emissions really be attributed to SOAEs?
Hear. Res., 80:141-145.

WAKE M., ANDERSON J., TAKENO S., MOUNT R.J. & HARRISON R.V. (**1996**)
Otoacoustic emission amplification after inner hair cell damage
Acta Otolaryngol. (Stockh.), 116:374-381.

WALSH R.M., HACKNEY C.M. & FURNESS D.N. (**1998**)
Hair-bundle degeneration and possible regeneration in the guinea-pig utricle following gentamicin-induced damage
Br. J. Audiol., 32 (2):71.
WHEELER L.J. & DIXON E.D.D. (**1952**)
The determination of the threshold of hearing
J. Laryng., 66:379-395.
WHITEHEAD M.L., BAKER R.J. & WILSON J.P. (**1989**)
The bilateral symmetry and sex asymmetry of spontaneous otoacoustic emission (SOAE) incidence in human ears
Br. J. Audiol., 23 (2):149.
WHITEHEAD M.L., KAMAL, N., LONSBURY-MARTIN B.L. & MARTIN G.K. (**1993**)
Spontaneous otoacoustic emissions in different racial groups
Scand. Audiol., 22:3-10.
WIER C.C., JESTEADT W., GREEN D.M. (**1977**)
Frequency discrimination as a function of frequency and sensation level
J. Acoust. Soc. Am., 61(1):178-184.
WIER C.C., PASANEN E.G. & MCFADDEN D. (**1988**)
Partial dissociation of spontaneous otoacoustic emissions and distortion products during aspirin use in humans
J. Acoust. Soc. Am., 84(1):230-237.
WILSON J.P. (**1980**)
Evidence for a cochlear origin for acoustic re-emissions, threshold fine-structure and tonal tinnitus
Hear. Res., 2:233-252.
WINKEL O., HANSEN M.M., KAABER K. & ROZARTH K. (**1978**)
A prospective study of gentamicin ototoxicity
Acta Otolaryngol., 86:212-216.
WOOD P.J., IOANNIDES-DEMOS L.L., LI S.C., WILLIAMS T.J., HICKEY B., SPICER W.J., HOOPER R.E. & MCLEAN A.J. (**1996**)
Minimisation of aminoglycoside toxicity in patients with cystic fibrosis
Thorax, 51:369-373.
YIN T.C.T. (**1995**)
Audition
In *Neuroscience in Medicine* (ed. Conn P.M.)
J.B. Lippincott Company, Philadelphia, pp. 485-499.
ZENNER H.P. (**1986**)
Motile responses in outer hair cells
Hear. Res., 22:83-90.
ZENNER H.P., ZIMMERMANN U. & SCHMITT U. (**1985**)
Reversible contraction of isolated mammalian cochlear hair cells
Hear. Res., 18:127-133.
ZHOU B. (**1995**)
Auditory filter shapes at high frequencies
J. Acoust. Soc. Am., 98(4):1935-1942.

ZIMMERMANN M. (**1989**)
The nervous system in the context of information theory
In *Human physiology, 2nd ed.* (eds. R.F. Schmidt & G. Thews)
Springer-Verlag, Berlin, pp. 166-173.
ZOROWKA P.G., SCHMITT H.J. & GUTJAHR P. (**1993**)
Evoked otoacoustic emissions and pure tone threshold audiometry in patients receiving cisplatinum therapy
Int. J. Pediatric Otorhinolaryngol., 25:73-80.
ZWICKER E. (**1970**)
Masking and psychological excitation as consequences of the ear's frequency analysis In *The proceedings of the international symposium on frequency analysis and periodicity detection in hearing* (eds. R. Plomp & G.F. Smoorenberg)
Sijthoff, Leiden, pp. 376-396.
ZWICKER E. (**1986a**)
A hardware cochlear nonlinear preprocessing model with active feedback
J. Acoust. Soc. Am., 80(1):146-153.
ZWICKER E. (**1986b**)
"Otoacoustic" emissions in a nonlinear cochlear hardware model with feedback
J. Acoust. Soc. Am., 80(1):154-162.
ZWICKER E. (**1986c**)
Suppression and ($2f_1$-f_2)-difference tones in a nonlinear cochlear preprocessing model with active feedback
J. Acoust. Soc. Am., 80(1):163-176.

APPENDIX

6.1. THE PHYSICS BEHIND SOUND MEASUREMENT

The physics behind sound measurement involves basic scientific principles that are common to all hearing research. The following glossary defines the important technical terms and concepts that are relevant to the preceding text.

6.1.1. SOUND

A sound wave is a longitudinal pressure wave, propagated by oscillations of the particles of the medium through which the wave travels. Sounds can be quantitatively represented in terms of the intensity and frequency components of individual waveforms, and the phase relationships between overlapping waveforms, which can be separated by Fourier analysis.

6.1.2. AMPLITUDE

The amplitude of a sound (a) is measured as the maximum distance moved by a particle from rest, perpendicular to the direction of the travelling wave. Simple harmonic motion is the oscillation of pressure following the pattern of a sine wave, and the sound produced is referred to as a pure tone. One cycle of simple harmonic motion refers to one complete rotation through 360°.

Where: Displacement = $a.\sin.\phi$

and: ϕ = Angular velocity (ω). Time (t)

Thus: Displacement = $a.\sin.\omega.t$

To find the average pressure throughout a cycle and account for the negative component of displacement, the root-mean-square (RMS) is calculated (otherwise known as the effective value), with respect to the prevailing ambient pressure.

For pure tones: RMS pressure = $0.707.a\,(N/m^2)$

6.1.3. WAVELENGTH

The wavelength of a sound (λ) is measured as the distance between any two corresponding points on two adjacent waves, for example between two successive points of maximum longitudinal compression. Standing waves are an interaction between the original and reflected waves so that reinforcement and cancellation occur at 'stationary' positions, which are dependent on the wavelength and distance from the boundary. The phase relationship of a sound refers to the number of degrees of delay of superimposed waves with respect to each other at a fixed point in time.

6.1.4. FREQUENCY

The frequency of a sound (f) refers to the rate of particle oscillation. More specifically, the frequency of a pure tone is a measure of the number of complete cycles made by a sine wave in unit time (number of cycles per second = Hz). An octave is the interval between two pure tones when their frequencies are in a ratio of 2:1. Complex tones are complicated waveforms that are composed of more than one frequency, and can be either periodic or non-periodic.

Periodic waveforms have a stable repeated pattern of fluctuation referred to as a fundamental frequency. Integral multiples of the fundamental frequency are referred to as harmonic frequencies (i.e. if the fundamental = n Hz, the second harmonic = 2n Hz, etc.). Fourier analysis

mathematically transforms complex periodic waveforms into the constituent pure tone frequency spectrum.

Non-periodic waveforms have random fluctuations referred to as noise. White noise is a continuous frequency spectrum of constant pressure. Narrow band noise is $^1/_3$ octave of constant pressure centred on one frequency of interest.

6.1.5. VELOCITY

The velocity of a sound (v) is a measure of the distance travelled with time in a given direction through a medium.

Where: $v = f.\lambda$ (m/s)

As longitudinal waves travel by a series of particle compressions (condensation) alternated with expansions (rarefraction) the velocity depends on the density and elasticity of the medium.

Where: v = square root of [E (modulus of elasticity) / ρ (density)]

The speed of sound in air (c) = 20.05.square root of absolute temperature (T)

When: T = 273.15 K, c = 331 m/s.*

6.1.6. IMPEDANCE

The acoustic impedance of a medium (Z) refers to the opposition of particles to the passage of sound. Acoustic impedance is measured as the ratio of the effective sound pressure to the effective particle velocity at a point in the progressive plane of a sound wave. Sound waves at a boundary between media of different impedance are refracted (transmitted)

---

* Speed of sound in air is approximately 340 m/s at standard temperature and pressure.

and reflected, to varying degrees depending on the angle of incidence. Acoustic impedance further depends on the prevailing temperature and atmospheric pressure. The inverse of impedance is compliance.

Where: $Z = \rho.c$

6.1.7. ATTENUATION

The attenuation of sound as it travels through a medium is calculated as the exponential loss of intensity with distance. Attenuation is due to either deviation (through divergence, scattering, diffraction, etc.) and / or absorption.

6.1.8. INTENSITY

The intensity of a sound refers to the magnitude of vibrational disturbance. More specifically intensity is a measure of the rate at which energy is transmitted through a specified area (which is perpendicular to the direction of propagation of the sound). Sound intensity is dependent on the systematic relationship between: displacement; velocity; and acceleration of media particles; along with the difference in pressure. When a sound wave radiates from a source the sound pressure is in phase with the particle velocity, but both are 90° in advance of particle displacement.

Where: Intensity = RMS pressure^2 / $\rho.c$

and: Pressure = Force / Area (N/m^2, or Pa)

Because the range of intensities from the faintest audible to painful sound is approximately one million-fold, for convenience, intensity is typically compressed into a logarithmic scale (and magnitudes are expressed in terms of ratios relative to some reference value). If an intensity is 10-fold

greater than the reference intensity the logarithm would be 1, the ratio being designated as 1 bel (after Alexander Graham Bell, 1847-1922). A ratio of 100-fold is 2 bels, and 1000-fold is 3 bels. To achieve a unit closer to the minimum discernible difference in intensity: 1 bel is divided into 10 steps, each $^{1}/_{10}$ bel being one Decibel (dB). An increase of 1 dB is thus equivalent to an increase in intensity by a factor of 1.26, whereas, 3 dB corresponds to a doubling in intensity and 10 dB corresponds to a 10-fold increase in intensity.

Where: Sound intensity level = $10 \log_{10}$ (Sound Intensity / Reference Intensity)

Intensity is proportional to the square of the pressure. Hence, to calculate the sound pressure level (dBSPL); the incident RMS pressure is taken as a ratio of the reference pressure and squared. An increase of 1 dB is thus equivalent to an increase in pressure by a factor of 1.12, whereas, 6 dB corresponds to a doubling in pressure and 20 dB corresponds to a 10-fold increase in pressure.

Therefore:

Sound pressure level = $10 \log_{10}$ (Sound Pressure / Reference Pressure)^2

Or:

Sound pressure level = $20 \log_{10}$ (Sound Pressure / Reference Pressure)

The reference pressure is 2×10^{-5} N/m^2, arbitrarily chosen and originally found as the smallest audible pressure at 1 kHz in young people with clinically normal ears (Sivian & White, 1933).

The spectrum level of a sound is equivalent to the intensity in dBSPL over a 1 Hz wide band. When measuring psychophysical performance, dBSPL is commonly converted to dBHL or dBSL. Where zero dBHL (hearing

level) is equivalent to the mean normative threshold in dBSPL at that frequency (EN 27566:1991; BS 2497:1992). Whereas, dBSL (sensation level) represents an intensity in dB with respect to the individuals' threshold.

6.2. TEST EQUIPMENT

6.2.1. INSTRUMENTS AND STANDARDISATION

All equipment intended for either direct or indirect patient contact complied with the *'Specifications for medical electrical equipment, part 1:1989 General requirements for safety'* (BS 5724:1989).

6.2.2. TEST EQUIPMENT

Fibre optic otoscope (Keeler, Vista 2.8V)

Hand-held tympanometer (Siemens, HandTymp DPU-411)

Clinical tympanometer (Kamplex, Middle-Ear Analyser KA 9)

Personal Computer hardware;

25 MHz, 386 IBM compatible (Pericom)

Personal Computer software;

DOS version 6.2, Windows version 3.1 (Microsoft), Word version 6.0, Excel version 5.0 (Microsoft), and Minitab version 10.0 (Minitab)

Oto-acoustic emissions hardware (serial number: 9492 DP 372 F/182);

card, analogue box and B-type adult probe (Otodynamics Limited)

Oto-acoustic emissions software (Otodynamics Limited, serial number 182);

ILO88 version v4.2 (1994) and ILO92 DP1 version v1.35 (1993)

Oto-acoustic emissions utility program (P. Bray, an_data.exe)

Clinical audiometers (Madsen OB822 and Kamplex KC-50 version 1.40);

both fitted with (Telephonics, TDH-39P) headphones (MX-51 cushions)

bone vibrator (Radioear, B-71) and insert earphone masker

Portable diagnostic audiometer (Kamplex KD-29, modified by PC Werth) fitted with (Telephonics, TDH-39P) headphones (Amplivox audiocups) bone vibrator (Radioear, B-71) and insert earphone masker

Compact Disc player (Sanyo, CDP-50A);

1 Bit DAC, digital anti-shock electronic skip protection, sound equalisation system

Notched-noise Compact Disc and IBM-compatible ROEX program (B.C.J. Moore, CD2)

Dual $^{1}/_{3}$ octave / precision equalizer (Alesis, M-EQ 230)

Audiometer interface amplifier (D.F. Heaton, project no. 602)

Sweep / Function generator (Thurlby-Thandar, 2 MHz TG230)

Etymotics insert earphones (ER-2 tubephones)

Foam ear plugs for hearing protection, size 5-13 (Arco, EAR 02302) and a 3-way punch

Foam ear-tips for Etymotics (ER1-14A)

Sterile alcohol surface wipes (Seton Prebbles Ltd., Sterets alcowipe)

6.2.3. CALIBRATION EQUIPMENT

Precision sound level meter (B&K, Type 2235), fitted with an input stage (B&K, Type ZC-0020)

Pre-polarised condenser microphones, free-field $^1/_2$" B&K):

Types 4176 & 4191

Type 4134, fitted to a Zwislocki occluded-ear simulator, with a Teflon microphone gasket (Knowles Electronics Inc., Types DB-100, and DB 009 respectively)

Pre-polarised condenser microphones, closed-field 1" cartridge (B&K):

Type 4145, fitted to a 2 cm^3 coupler (B&K, Type DB-0138)

Type 4144, fitted with an adapter ring to an artificial ear with guard ring adapter (B&K, Types DB-0111, 4152, and DB-1021 respectively) either microphone via a $^1/_2$" to 1" adapter (B&K, Type DB-0962)

Artificial mastoid (B&K, Type 4930) fitted with 2 adapters (B&K, Types JP-0028 & JJ-2614)

Octave band-pass filter set (B&K, Type 1618) with cable (B&K, Type AO-0037)

Sound level calibrator; 1 kHz piston-phone @ 94 dBSPL (B&K, Type 4230)

Oscilloscope; 100 MHz, 2 channel (Hewlett-Packard, 54600A)

Dynamic signal analyser (Hewlett-Packard, 35665A)

6.2.4. EXPORT OF RAW OAE DATA

The SOAE data files (identified by an '.ana' extension in the ILO92 software) were processed by running a utility program ('an_data.exe') from within the same computer sub-directory, to produce a single file ('analyser.csv'). The DP-gram data files (identified by a '.dpg' extension in the ILO92 software) were processed by opening the file, selecting 'numerical analysis' and re-saving as a 'spreadsheet file', resulting in a single file (identified by a '.spr' extension and placed within the 'logdata' sub-directory) limited by tab and comma.

AMINOGLYCOSIDE / ANTIBIOTIC DOSAGE

NAME:

D.O.B.:

CODES:

gentamicin = a	*ceftazidime = e*	*imperacin = i*
netilmicin = b	*piperacillin = f*	*flucloxacillin = j*
amikacin = c	*azlocillin = g*	*ampicillin = k*
tobramycin = d	*aztreonam = h*	*colomycin = l*

* = not known

									At 3rd dose & 2nd week			
DRUG 1	DRUG 2	**START**	**END**	NO.	**LEVEL**	**FREQ.**	**BODY**	DOSE/KG	**SERUM LEVELS (mg/l)**			
		DATE	**DATE**	DAYS	**(mg)**	**times/day**	**WEIGHT**	/DAY	**PRE-1**	**POST-1**	PRE-2	POST-2

6.4. Information about some new hearing tests

Thank you very much for considering to take part in this study, in which we hope to test some new ways of measuring hearing. In this information sheet we have included details about why we want to develop these tests, why we would like you to help us and what they involve.

Why are these new hearing tests important?

As someone with Cystic Fibrosis (CF) or a parent of a child with CF, you know that antibiotics are often given to treat chest infections. Sometimes intravenous antibiotics, such as gentamicin or tobramycin, are given which have to be closely monitored by taking blood levels (gentamicin levels). The levels are taken to ensure that enough gentamicin is given to clear the infection, and also ensure that any side effects, such as dizziness or difficulty with hearing, are kept to a minimum.

We are developing some new ways of looking at hearing that are able to pick up very early changes in hearing ability. These tests are being developed jointly with the Department of Medical Physics at Leicester Royal Infirmary and a number of other hospitals. We hope that these tests will enable us to monitor any side effects of treatment, keep any side effects to a minimum, and ensure that you or your child receive/s the best kind of treatment for infections at the most effective dose.

How can you help?

We would like you to fill in a questionnaire about your (or your child's) general health, and any hearing problems that you have noticed. There will be someone available who will be happy to help you fill this in. The information on the form will be confidential and only used for the purposes of the study. We would then like to perform the hearing tests when you come to the CF clinic. The tests are very safe and easy to perform. The hearing tests take between 10 to 20 minutes each and we would like to do two of the tests at a visit. We hope not to keep you for more than 40 to 60 minutes at any one time. We would like to repeat these tests when you come to your next clinic visit.

What are the tests and what do they involve?

Ear examination and Middle-ear function test

We will have a quick look in your ears. Then an ear piece will be placed against your ear and you will hear a low musical tone and feel a slight pressure for a short time. This will enable us to check that your eardrum is moving correctly and that you have no middle ear problems.

Pure tone threshold tests

This is a simple test where you are asked to listen to very quiet sounds over headphones to see how sensitive your hearing is. The sounds used cover a range of low frequencies (such as bass sounds often used to give music its beat) to high frequencies (such as treble sounds that can sound like bird song). In this test you will simply be asked to press a button when you can hear the sound coming from the headphones.

Frequency resolution tests

The ear is very good at separating different frequencies present in the sounds we hear. For example, we are able to listen to one person talking even if we are in a roomful of other people talking. In this test we are looking at how well you can sort out single frequencies in the presence of other 'mixed' frequencies. In this test you will simply be asked to press a button when you can hear the single frequency sound above the background noise of 'mixed' frequencies.

Oto-acoustic emissions tests

This is a test that is very easy to do. It involves sitting quietly and listening to some clicks and musical tones from a small ear piece and the machine does the rest. When we hear noise, the inner ear actually produces a sound that can be detected by a small microphone in the ear piece, and in this way we can assess how well the inner ear is working.

6.5. Consent form

I have read the accompanying information sheet and I am happy for myself / my child to be involved in this study of hearing ability. I understand that I can withdraw myself / my child from the study at any time. I understand that this study will not affect the way I / my child, am / is treated in any way.

Name of patient .....

Signature of patient .....

Signature of parent / guardian (if patient is under 18 years old)

.....

Date .....

Thank you

Prof. K.L. Woods, Dr. M. Mulheran, Steven Burr.

6.6. Hospital Hearing Questionnaire

The purpose of our research is to develop new hearing tests that will enable the early detection of side-effects due to certain medications. This questionnaire will enable us to better interpret the results from the hearing tests. The results from this research will also help doctors make decisions on the medication for your treatment.

Thank you for agreeing to take part in our survey. Please answer all questions carefully; if you are unsure about any question(s) leave them unanswered. Please do not hesitate to mention any questions or further information that you think may be relevant. All the information you give will be kept completely confidential and will only be seen by workers directly involved in the study.

Section 1. General Information

1.1 Name:

1.2 Date of Birth: Age:

1.3 Male / Female

1.4 Telephone number:

1.5 Occupation:

Section 2. General Health

2.1 How old were you when cystic fibrosis was diagnosed?

2.2 Do you have asthma? Yes / No

2.3 Do you have diabetes? Yes / No

2.4 Do you have epilepsy? Yes / No

2.5 Do you have any problems with your general circulation, for example do you often have cold hands or feet even when it is not very cold?
Yes / No

2.6 Have you ever received a very sharp knock or blow to the head that resulted in a temporary loss of consciousness (i.e. concussion)?
Yes / No

Medical Research Hearing Questionnaire

The purpose of our research is to develop new hearing tests that will enable the early detection of side-effects due to certain medications. To this end we need volunteers who have not been exposed to these medications and who have otherwise normal hearing. These questionnaires will enable us to select individuals who are suitable for having their hearing tested and compared with the patients receiving treatment.

Thank you for agreeing to take part in our survey. Please answer all questions carefully; if you are unsure about any question(s) leave them unanswered. Please do not hesitate to mention any questions or further information that you think may be relevant. All the information you give will be kept completely confidential and will only be seen by workers directly involved in the study.

Section 1. General Information

1.1 Name:

1.2 Date of Birth: Age:

1.3 Male / Female

1.4 Telephone number:

1.5 Occupation:

Section 2. General Health

2.1 Overall, would you say you were generally healthy?
Yes / No

2.2 Do you have asthma? Yes / No

2.3 Do you have diabetes? Yes / No

2.4 Do you have epilepsy? Yes / No

2.5 Do you have any problems with your general circulation, for example do you often have cold hands or feet even when it is not very cold?
Yes / No

2.6 Have you ever received a very sharp knock or blow to the head that resulted in a temporary loss of consciousness (i.e. concussion)?
Yes / No

2.7 Have you ever been ill with any of the following:

Meningitis?	Yes	/	No
German measles (Rubella)?	Yes	/	No
Chicken pox?	Yes	/	No
Scarlet fever?	Yes	/	No
Mumps?	Yes	/	No
Whooping cough?	Yes	/	No
Influenza?	Yes	/	No
Tuberculosis?	Yes	/	No
Jaundice?	Yes	/	No

2.8 Were you ever in intensive care as a baby?

Yes / No

Section 3. Medication

3.1 Have you ever taken aspirin regularly? Yes / No

3.2 Have you ever been prescribed medication for a:

Ear problem?	Yes	/	No
Skin problem?	Yes	/	No
Mouth infection?	Yes	/	No
Kidney problem?	Yes	/	No
Heart problem?	Yes	/	No

3.3 Are you allergic to penicillin? Yes / No

3.4 Have you ever been prescribed any aminoglycoside antibiotics?
These often have names ending in -micin or -mycin.

Yes / No / Not sure

Have you ever been prescribed any of the following:

Gentamicin?	Yes	/	No	/	Not sure
Netilmicin?	Yes	/	No	/	Not sure
Amikacin?	Yes	/	No	/	Not sure
Neomycin?	Yes	/	No	/	Not sure
Tobramycin?	Yes	/	No	/	Not sure
Streptomycin?	Yes	/	No	/	Not sure

3.5 Are you taking any prescribed medication at the moment?
If so please give details below:

3.6 Are you taking any over the counter medication at the moment?
If so please give details below:

Section 4. Noise Exposure

4.1 Below is a list of activities that you may have been involved in. If you have, put in the space beside it how many hours a week or month on average you have spent in these activities.

	Hours a week or month (please specify which)	
	At the moment	**In the past**
Learning to play a musical instrument?	hrs/	hrs/
Playing in a band or orchestra?	hrs/	hrs/
Attending discos or pop / rock concerts?	hrs/	hrs/
Using stereo headphones?	hrs/	hrs/
Listening to amplified music?	hrs/	hrs/
Near noisy machinery, engines or equipment	hrs/	hrs/

4.2 Have you ever been exposed to a very loud noise, such as a shotgun going off? Yes / No

Section 5. Hearing Problems

5.1 Have you ever had an ear or hearing problem due to a sporting activity? Yes / No

5.2 Do your ears seem to get 'blocked up' every time you get a cold? Yes / No

5.3 Do you often have earache? Yes / No

5.4 Have you ever had a discharge from your ears? Yes / No

5.5 Have you ever seen a doctor about your ears or hearing? Yes / No

5.6 Have you ever had an ear operation? Yes / No

5.7 When people are talking to you do you sometimes have difficulty being able to tell what is being said? Yes / No

5.8 If you answered Yes to the previous question, how often would you say this was?

Not very often.
Quite often.
Most of the time.
Nearly all, or all of the time.

5.9 If you answered Yes to question **5.7**, does what is being said seem worse if there is any other conversation or noise going on in the background? Yes / No

5.10 Have you ever had bouts of light-headedness?
Yes / No

5.11 Have you ever had bouts of vertigo (i.e. sensation of movement when stationary)? Yes / No

5.12 Many people sometimes say that they can hear noises in their head or in their ears. These noises can sound like a television or a radio not tuned in right, or like a whistling noise or sometimes a pulsing noise. This sensation is often referred to as tinnitus.

Have you ever had tinnitus? Yes / No

5.13 If you answered Yes to the previous question:

How often would you say that you get tinnitus?
Hardly ever.
After listening to loud sound or music.
At other times, lasting for more than ten minutes or so.
It seems to be there all the time.

When you have tinnitus which ear does it seem to be coming from?
Left / Right / Both together / Both at separate times

When you have tinnitus how loud would you say it seemed to be?
Quiet / Not very loud / Quite loud / Very loud

5.14 Is there anyone in your immediate family who was born deaf or developed hearing problems at an early age?
Yes / No

If so, how are they related to you, and what is their hearing problem?

Section 6. Any Comments?

If you have any further comments please write them overleaf.

Thank you very much for taking the time to complete this questionnaire.