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OTOACOUSTIC EMISSION (OAE)-BASED MEASUREMENT OF THE FUNCTIONING OF HUMAN COCHLEA AND THE EFFERENT AUDITORY SYSTEM

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Abstract

The discovery of otoacoustic emissions (OAE) has advanced our understanding of cochlear mechanics and the efferent auditory system. OAE are sounds generated within normal cochlea either spontaneously or in response to stimulation. The ability to measure OAE non-invasively, objectively and quickly makes a powerful tool to probe cochlear mechanics. Stimulation of the efferent auditory system causes changes in cochlear amplification processes and hence changes characteristics of OAE. Contralateral acoustic stimulation, commonly called OAE suppression, provides an index of the efferent auditory system (specifically, medial olivocochlear bundle) functioning. OAE is also a sensitive tool to demonstrate subtle changes in cochlear functioning caused by various pathological (e.g., noise exposure, aspirin toxicity, etc.) and non-pathological (e.g., posture, efferent stimulation) factors. Although OAE are frequently used in both clinic and laboratory, their generation mechanism was not clearly understood until recently. It is currently accepted that distortion product otoacoustic emissions (DPOAE) are composed of two separate components, named wave- and place-fixed emissions. They not only arise from two different cochlear locations but also from two fundamentally different processes. Wave-fixed components arise from distortion sources and manifest a phase that is almost independent of frequency, where as, place-fixed components arise from reflection sources and have a phase that increases systematically with frequency.

The overall aim of the work presented in this thesis was to use various OAE methods to examine cochlear function and the efferent auditory system. A related objective was to substantiate the functional relevance of the efferent auditory system in speech-in-noise perception, in order to address the clinical significance of measuring OAE suppression. Cochlear functioning was potentially manipulated by three treatments separately: one extrinsic (electromagnetic radiation exposure from mobile phone) and two intrinsic (posture and efferent activation). Potential changes in auditory function due to mobile phone exposure were evaluated in a within-subject study in a double-blind design (n=35). A comprehensive examination of the auditory system was conducted using audiometry, OAE and auditory event related potentials (ERP). The second experiment used mechanism-based DPOAE to investigate posture-induced changes in cochlear functioning (n=15). Similar DPOAE measurements were performed to evaluate the effect of contralateral acoustic stimulation on cochlear functioning (n=14). The last experiment examined the relationship between contralateral suppression of transient evoked otoacoustic emissions (TEOAE) and recognition of speech in noise (n=13).

Results indicate that (i) acute exposure to mobile phone radiation does not cause any significant changes in auditory functions measured by TEOAE suppression, DPOAE or ERP (however, there were changes in auditory thresholds at 6 and 8 kHz), (ii) posture-induced cochlear changes and contralateral acoustic stimulation cause significantly greater reduction in place-fixed components than wave-fixed components, and (iii) the efferent auditory system plays an anti-masking role in speech-in-noise recognition. It appears that wave- and place-fixed components are differentially sensitive to changes in cochlear functioning. Collectively, the present results provide emerging empirical support for the need to separate the wave- and place-fixed components in DPOAE measurements. Because of inherent differences in the generation of wave- and place-fixed components, it is suggested that the separation of the components may improve the efficiency of DPOAE-based measures of cochlear dysfunction and also, of the efferent auditory system function.

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List of Abbreviations

ABR	Auditory brainstem responses
AEP	Auditory evoked potentials
AMLR	Auditory middle latency responses
ART	Acoustic reflex thresholds
BBN	Broadband noise
CAS	Contralateral acoustic stimulation
CS	Contralateral suppression
DPOAE	Distortion product otoacoustic emission
EMF	Electromagnetic fields
ERP	Event related potentials
FAAF	Four alternative auditory features
GSM	Global system for mobile communications
HTL	Hearing threshold level
ICNIRP	International commission on non-ionizing radiation protection
IFFT	Inverse fast Fourier transforms
OCB	Olivocochlear bundle
MEMR	Middle ear muscle reflexes
OAE	Otoacoustic emissions
OHC	Outer hair cells
RF	Radio frequency
SAR	Specific absorption rate
SNR	Signal to noise ratio
SOAE	Spontaneous otoacoustic emissions
TEOAE	Transient evoked otoacoustic emissions
UMTS	Universal mobile telecommunication system

CHAPTER 1

Introduction

The functioning of the cochlea is most commonly evaluated by otoacoustic emissions (OAE). OAE are sounds generated within the normal cochleae either spontaneously or evoked by stimulation (Kemp, 1978). There are two basic types of OAE: (i) spontaneous emissions (SOAE), and (ii) evoked emissions (EOAE). SOAE occur in absence of external stimulation, whereas EOAE occur during or after external stimulations. There are several subclasses of EOAE based primarily on the stimuli used to evoke them. These include: (i) transient/click evoked otoacoustic emissions (TEOAE); (ii) distortion product otoacoustic emission (DPOAE); and (iii) stimulus frequency emissions (SFOAE). DPOAE is a type of OAE that can be evoked by two pure tones presented simultaneously. Histological examination has revealed that outer hair cell damage lead to the reduction of OAE amplitudes, thus verifying the involvement of outer hair cells in the generation of OAE (Brown, McDowell, and Forge, 1989).

In recent years, it is accepted that multiple mechanisms and/or sources contribute to the generation of DPOAE. At least, two separate mechanisms are thought to contribute to the generation of DPOAE: (i) nonlinear distortion and (ii) linear coherent reflection (Shera and Guinan, 1999; further details in Chapter 2). Distortion and reflection mechanisms are also called wave- and place-fixed components respectively (Knight and Kemp, 1999, 2000, 2001). With appropriate measurement techniques DPOAE recorded in the ear canal can be separated into wave- and place-fixed components. The separation of components into wave- and place-fixed components provides important insight into generation mechanisms of DPOAE. By explicitly identifying and classifying the differences between wave- and place-fixed components, DPOAE would provide an improved interpretive framework that has the potential to enhance the scientific and clinical utility in several important ways, for instance, measurement of the efferent auditory system function. As a consequence of their different origin sources (Shera and Guinan, 1999), it is presumed that the wave- and place-fixed components manifest different dependencies on cochlear functions and pathologies. Measurement of

components of DPOAE would thus offer windows of opportunity to more appropriately assess subtle changes in cochlear functioning due to various intrinsic and extrinsic factors. It may prove to be a more sensitive and specific indicator of cochlear mechanisms.

OAE can be easily applied to study the efferent auditory system. It is well known that contralateral acoustic stimulation of OAE usually induces changes in OAE parameters in the ipsilateral ear. The efferent auditory system specifically the medical olivocochlear system (MOC) modulates these changes (see Guinan, 2006, for review). By carefully measuring the changes in OAE the functioning of the efferent system can be evaluated. In this thesis, the knowledge of wave- and place-fixed components is applied to study and develop a sensitive DPOAE-based assay for suppression measurements.

The present thesis aimed at the measurement of cochlear and efferent auditory system functioning in the context of mechanism of generation of OAE. This translational research, specifically, explores the possibility of using wave- and place-fixed components in an attempt to evaluate changes in cochlear and efferent auditory system functioning. It also uses the traditional OAE measures of cochlear functioning (such as, TEOAE and composite DPOAE). Attempts were made to evoke change in cochlear functioning via two non-invasive ways: (i) radiation from mobile phones, and (iii) body position. While body position is a well-known factor to induce changes in cochlear functioning, the potential effect of mobile phone radiation on cochlear functioning is not well known. The efferent system functioning was measured by traditional OAE suppression and via novel DPOAE techniques. The functional relevance of the efferent auditory system in speech perception in noise was also examined.

The potential changes in cochlear functioning due to mobile phone radiation were measured using more traditional OAE methods. Changes due to efferent activation and posture-induced changes were evaluated using more contemporary OAE methods such as, wave- and place-fixed emissions. Also, changes due to mobile phone radiation in other parts of the auditory system; for example the efferent and central auditory pathways were also examined to a limited degree.

The study based on mobile phone radiation was part of the European Project EMFnEAR "Exposure to Universal Mobile Telecommunication Systems (UMTS) Electromagnetic Fields: Study on Potential Adverse Effects on Hearing", European Commission, DG Health and Consumer Protection, Public Health and Risk Assessment, Work Plan 2004, Commission decision 25 February 2004 2004/192/EC (Grant agreement No 2004127, 2004-2007).

1.1. Organization of the thesis

This thesis is organised into seven chapters. The chapters are arranged based on measurement techniques and novelty, rather than the order of completion of the actual experiments. Chapters 3, 4, 5 and 6 are the main body of experiments and are written as self-contained manuscripts.

The thesis work started with experimentation using a variety of tests (as in Chapter 3), and then examines the relative usefulness of DPOAE, wave- and place-fixed components in assessing cochlear functioning (Chapter 4) and efferent effects (Chapter 5). Based on the findings (from Chapter 4 and 5) that place-fixed components are relatively more sensitive to changes in cochlear mechanisms, the last experiment (in Chapter 6) used TEOAE (predominantly place-fixed OAE) to evaluate the function of efferent auditory system in speech-in-noise perception. The thesis work evolved from generic tests of auditory function and progressed towards more specific tests of cochlear mechanisms and efferent effects.

The first (this) chapter introduces the thesis and highlights the contribution to knowledge. It also lists research output of the thesis in terms of conference presentations and publications.

Chapter 2 presents the necessary background of DPOAE generation and provides a comprehensive review of generic changes in the auditory system due to mobile phone radiation, and a review of cochlear functioning specifically in the context of body position and efferent activation. The functional relevance of efferent activation in speech perception is also reviewed. Finally, chapter 2 defines the specific research aims.

Chapter 3 describes the mobile phone experimentation and discusses the findings related to changes in auditory functions due to mobile phone exposure. This chapter used a variety of methods from OAE to evoked potentials.

Chapter 4 examines the changes in the components of DPOAE when changes in cochlear functioning are induced by manipulating body position.

Chapter 5 describes measurement of changes in cochlear functioning due to efferent activation using novel DPOAE methods (i.e., wave- and place-fixed components).

Chapter 6 presents a basic experiment that aims at evaluating efferent auditory system functioning via contralateral suppression of TEOAE and it also addresses the functional relevance of such a change for speech perception.

Chapter 7 provides a comprehensive summary of the entire experimental work and presents a simple model of changes in cochlear functioning with regard to wave- and place-fixed emissions. It also lists the possible avenues for future research

1.2. Contribution to knowledge

Each of the experiments in this thesis depict certain novel aspects related to cochlear and efferent functioning or tests used to measure these functions. For instance, Chapter 3 is the first of its kind to evaluate the potential effects of UMTS phone radiation on auditory functions in a comprehensive fashion. Chapter 4 and 5 display the novel idea of using wave and place-fixed DPOAE measures to probe cochlear and efferent mechanisms. Chapter 5 also provides systematic examination of the effect of intra-cranial pressure (ICP) induced by body position on the cochlea. Chapter 6 used a complex speech perception task in a non-conventional noise background (speech-shaped noise) to substantiate the functional relevance of efferent auditory system for speech perception.

The thesis first examines the potential changes in the auditory system and specifically cochlear functioning due to mobile phone exposure in a more comprehensive fashion by including a wide range of tests from audiometry to auditory evoked potentials. This

establishes the evidence base for the potential effects of radiation exposure from the latest generation of mobile phones.

One of the important contributions of this thesis is establishing the trend to evaluate cochlear functioning by using mechanism-based OAE measurements. This novel technique is applied to study the changes in cochlear functioning due to body position and efferent suppression measurements, separately. The idea of applying DPOAE component measurement to study efferent mechanisms is novel and provides a unique opportunity to resolve some of the long-standing scientific issues. The other contribution of this thesis, to a lesser extent though, is replicating the functional relevance of efferent activation in speech perception using a different speech in noise recognition testing method. The speech perception task was made difficult with the use of speech-shaped noise. Indirectly, this thesis also provides partial evidence on the source of DPOAE fine structure.

This thesis highlights the importance of and provides scientific evidence for measurement of components of DPOAE to evaluate cochlear and efferent auditory system mechanisms. This also expands our current understanding and knowledge on suppression measurements.

The clinical contribution of this translational research is to improve early and sensitive diagnostic tests of hearing impairment, improve aetiological specificity and enhance the power of current DPOAE-based measures of the cochlear and efferent auditory system. Overall, by introducing the wave- and place-fixed emissions as a tool to evaluate changes in cochlear functioning, DPOAE could provide new insights on the measurements of cochlear and efferent mechanisms. It highlights promising areas of research in both hearing science and clinical audiology; e.g., from maturation of cochlear mechanisms and threshold estimation to monitoring of subtle changes in the cochlea.

Conference presentations and publications

Mishra SK, Lutman ME. Effect of contralateral acoustic stimulation on DPOAE generation mechanisms in humans. Hearing Research (under review).

Parazzini M, Sibella F, Lutman ME, Mishra S, Moulin A, Sliwinska-Kowalska M, Thuroczy G, Tavartkiladze G, Bronyakin S, Uloziene I, Uloza V, Ravazzani P. Effects of UMTS cellular phones on human hearing: results of the European project 'EMFNEAR'. Radiation Research 2009; 172: 244-251.

Abdala C, Mishra SK, Williams T. Considering distortion product otoacoustic emission (DPOAE) fine structure in measurements of the medial olivocochlear reflex. J Acoust Soc Am. 2009; 125:1584-94.

Mishra SK, Lutman ME. Effects of mobile phone radiation on auditory cortical functioning. International Conference on Hearing Therapies, 25- 27 Sept 2007. USA. Supported by conference grant from NCRAR, US Dept, of Veteran Affairs.

Mishra SK, Lutman ME. Influence of the medial olivocochlear system on distortion and reflection components of DPOAE. International Congress of Audiology, 3-7th Sept 2006, Innsbruck, Austria. Supported by student conference grants of the International Society of Audiology.

Mishra SK, Lutman ME. Influence of the auditory efferent system on speech perception in noise: physiological and behavioural evidence. International Hearing Research Conference, 16- 20th Aug 2006, USA. Supported by RNID Knowledge transfer grant.

Lutman ME, Mishra SK, Moulin A, Parazzini M, Sliwinska-Kowalska M, Tavartkiladze G, Uloza V. Effects of mobile phone electromagnetic fields on the human auditory system. NHS, 31 May- 3rd June 2006, Como Lake, Italy.

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CHAPTER 2

LITERATURE REVIEW

The peripheral auditory system receives acoustic input and acts as a gateway to the auditory centres in the brain. Mechanisms of the peripheral system, especially the cochleae are not passive. Cochlea modifies the input in a specific and complex way. Normal cochleae generate acoustic signals, called otoacoustic emissions (OAE). OAE can be spontaneous or evoked and recorded in the ear canal using a small, sensitive, low-noise microphone (Kemp, 1978). There are two basic types of OAE: (i) spontaneous emissions (SOAE), and (ii) evoked emissions (EOAE). SOAE occur in absence of external stimulation, whereas EOAE occur during or after external stimulations. There are several subclasses of EOAE based primarily on the stimuli used to evoke them. These include: (i) transient/click evoked (TEOAE); (ii) DPOAE; and (iii) stimulus frequency emissions (SFOAE). However, the mechanism based taxonomy suggested by Shera and Guinan (1999) hypothesizes that TEOAE and SOAE are categorized as originating from linear reflection, whereas DPOAE are produced predominantly by non-linear distortion. Further DPOAE can be sub-divided into place- and wave-fixed components. Place-fixed emissions have increasing phase with frequency and arise due to variations in cochlear reflectance, while wave-fixed emissions have approximately constant phase across frequency and arise from distortion mechanisms (Knight and Kemp, 1999, 2000, 2001; Wilson and Lutman, 2006).

The following paragraphs provide a brief description of the anatomy of the cochlea. It is not intended to be detailed description of the cochlea. Figure 2.1 displays the classic view of the cochlea showing important anatomic features.

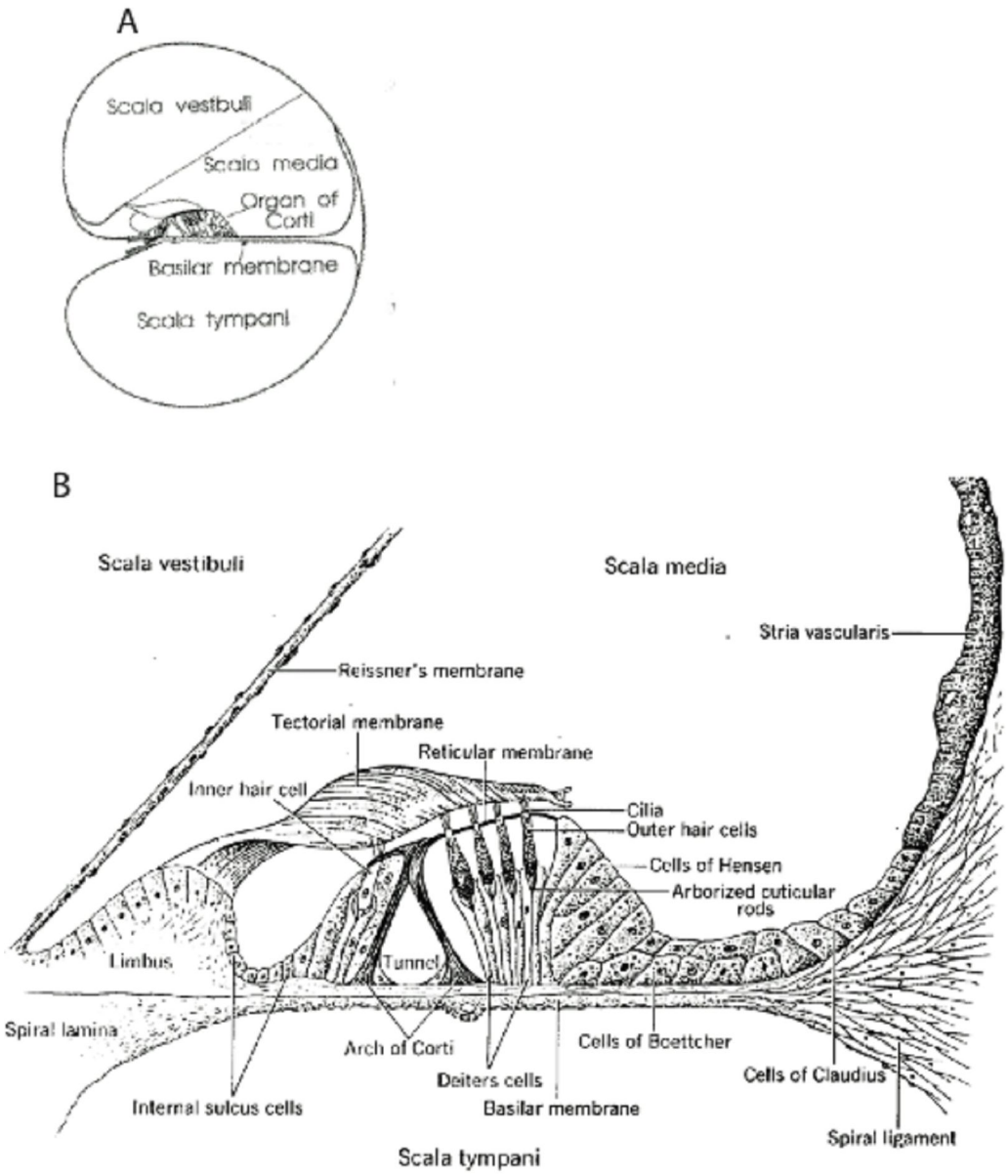

Figure 2.1. Classic view of the cochlea (adapted from Schuknecht, 1993) (A) Section through the cochlea (B) Cross-section through the organ of Corti.

The cochlea bears some resemblance to a common snail-shell. It has $2\frac{3}{4}$ turns. It forms the anterior part of the labyrinth, and is housed within the temporal bone. It consists of bony labyrinth curling around a central core modiolus, and is subdivided into three compartments; scala tympani, scala vestibuli and scala media. Scala tympani and scala vestibuli are filled with perilymph (high in sodium), whereas the other membranous compartment, scala media, is filled with endolymph (high in potassium). At the apex, perilymph of the scala vestibuli continues into scala tympani thorough a tiny passage called helicotrema. Scala tympani runs from medially to laterally (toward the stria vascularis) of three continuous structures; the spiral limbus, the basilar membrane and the spiral ligament and on the upper side of the Reissner's membrane. Endolymph makes direct contact with the specialized cells such as, hair cells, Hensen's cells, Claudius' cells. Some of the important structures within the cochlea are as follows;

Basilar membrane (BM)- The basilar membrane stretches from the tympanic tip of the osseous spiral lamina to the basilar crest. It is a stiff structural element that separates the scala media and the scala tympani.

Organ of Corti- Organ of Corti rests on the basilar membrane within scala media in the inner ear. It is composed of a series of epithelial structures placed upon the inner part of the BM. The more central of these structures are two rows of rod-like bodies, the inner or outer pillars of Corti. The bases of the rods are supported on the basilar membrane, and the inner and the outer rows incline toward each other, coming into contact above, forming a triangular tunnel, called Tunnel of Corti. On the inner side of the inner pillars is a single row of hair cells, and on the outer side of the outer pillars there are three or four similar cells, together with some supporting cells called Deiter's cells. The free ends of the outer hair cells occupy a series of apertures in a net like membrane called as Reticular membrane, and the entire organ is covered by the Tectorial membrane.

Hair Cells –The hair cells are short columnar cells. The inner hair cells (IHC) (3000-4000 in number) are arranged in a single row on the medial side of the inner rods, and each hair cell is supported by more than one rod. The free ends of the inner hair cells are encircled by a cuticular membrane, which is fixed to the heads of the inner rods. The outer hair cells (OHC) are 12000 in number and are nearly twice as long as the inner. The OHCs are arranged in three regular rows in the basal coil of the cochlea, and somewhat

irregular rows, in four, in the apical coil of the cochlea. The bottom of these cells is attached to the BM and they have hair like projections at the top of the cell known as stereocilia.

Stereocilia- The stereocilia are apical modifications of the cell. These are mechano-sensing organelles of hair cells, which respond to fluid motions or fluid pressure changes. The Stereocilia are composed of cytoplasm with embedded bundles of cross-linked actin filaments. Stereocilia resembles hair-like projections, and are arranged in bundles of 30-300. Within the bundle the stereocilia are often lined up in several rows of increasing height. The top of the sterrecilia are in contact with the Tectorial membrane.

Tectorial membrane- Covering the spiral organ of Corti is the Tectorial membrane, which is attached to the limbus laminae spiralis close to the inner edge of the vestibular membrane. This membrane partially covers the hair cells in organ of Corti and vibrates when fluid sound wave hit it. A structure known as Hardesty's membrane divides the subtectorial space into two compartments, once facing the surface of inner hair cells and other facing the surface of OHC.

Inside the cochlea, sound waves cause the BM to vibrate up and down. This creates a shearing force between the BM and the tectorial membrane, causing the hair cell stereocilia to bend back and forth. This leads to internal changes within the hair cells that create electric signals, which are then passed by the auditory nerves to the brain.

Since the cochlea is embedded in the temporal bone, the ability to non-invasively measure OAE that originate within it provides unique and illuminating access to this otherwise inaccessible structure. Despite the attendant extraordinary clinical and research implications, the mechanisms have not been entirely understood until recently. The goal of the vast majority of current OAE research is to increase and specify the amount of information available from OAE. OAE not only provide important information on cochlear mechanisms, but also have exceptional potential to study the efferent auditory system.

The following paragraph provides a brief introduction to the efferent auditory system pathway (primarily from Guinan, 2006).

Not only neurons carry information from periphery to the auditory cortex, neurons from the brainstem also contact hair cells. These neurons carry information from the brain to the ear and are called efferent neurons. The fibre tract containing the efferent fibres is known as the olivocochlear bundle (OCB). OCB constitutes a feedback loop, by which nerve impulses, thought to be inhibitory, reach the hair cells. This system uses acetylcholine as a neurotransmitter. The tract from the same side of the brain is called the uncrossed OCB and the tract from the opposite side of the brain is called the crossed OCB. There are two types of OCB; medal (MOCB) and lateral (LOCB). Figure 2.2 presents a schematic diagram of the OCB pathway to the right cochlea. Thick, myelinated MOC fibres to the right cochlea originate in the medial part of the superior olivary complex (SOC) on both sides and project through the vestibular nerve to the cochlea, where they innervate the OHC. Thin, unmyelinated LOC fibres to the right cochlea originate predominantly on the right (ipsilateral) side of the brain. Their axons also travel via the vestibular nerve, but LOC fibres innervate auditory nerve fibres under IHC. The OCB contacts on OHCs differ from those on IHCs. MOCB form large calyx-shaped contacts on the OHC cell body but LOCB form small button-like contacts on the afferent nerve fibres that contact IHC. MOC fibres are thick and myelinated, which allows both recording and electrical stimulation of MOC fibres. In contrast, LOC fibres are thin and unmyelinated, as a result it is difficult to stimulate or record their activity.

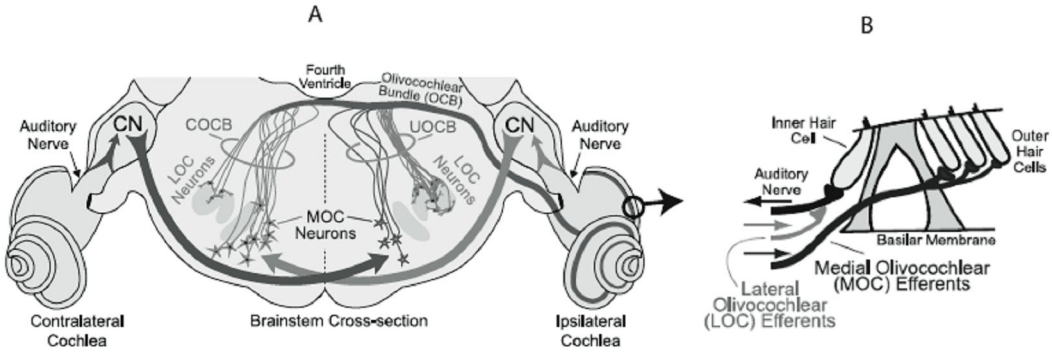

Figure 2.2. Shows diagram of the OCB pathway the right cochlea (from Guinan, 2006). (A) Transverse section of the brainstem of a cat showing MOC and LOC fibres. (B) Organ of Corti showing the main terminations of the OCB.

2.1. Components of DPOAE

Distortion is always generated when there is mechanical; non-linearity and OHC are mechanically non-linear. DPOAE, by definition, represent cochlear nonlinear responses, because they consist of new frequencies that are not present in the evoking stimuli (Kemp, 1979). They are produced through the nonlinear interaction of two closely spaced tones, usually called primaries, F1 and F2 (with F2>F1). They may appear at frequencies equal to 2F1–F2, 3F1–2F2, 4F1–3F2 (lower side band), and 2F2–F1, 3F2–2F1 (basal to the primary frequency place) and so on.

Theoretically, the generation of distortion products due to the interaction of the two primaries is spread over the entire basilar membrane; however, the major contributor for the DPOAE (at least for 2F1-F2 DPOAE) is at a region of about 1mm around the F2 characteristic place. Brown and Kemp (1983) provide evidence for this by adding a suppressor tone between F1 and F2, which effectively reduces the DPOAE amplitude. This generation site at the region of maximum overlap of the two travelling wave envelopes evoked by the two primaries is referred to as the F2 place. Since the DPOAE recorded in the ear canal consist of new frequencies (Fdp) that are not present in the eliciting stimuli, it is evident that there is at least another source at the Fdp characteristic frequency, in the form of the stimulus frequency emission (Brown and Gaskill, 1990; Gaskill and Brown, 1990, 1996). Recent studies have indicated this second source contributes to the generation of the apical components 2F1–F2, 3F1–2F2, 4F1–3F2 DPOAE (Knight and Kemp, 1999, 2000, 2001; Wilson and Lutman, 2006). Some studies have demonstrated this two-source (primary- and secondary-source) hypothesis by introducing a low intensity suppressor tone close to the DP frequency (Gaskill and Brown 1996; Heitmann *et al*., 1998). Stover, Neely, and Gorga (1996) supported the two-source hypothesis by using the latency of the DPOAE, which they argued to be an indirect measurement of the site of generation. The latency, in principle, should represent the sum of forward travel time of the stimulus to the generation site and the reverse travel time as the emission travels from this site back out of the cochlea, via the middle ear to the ear canal. Because the two sites are spatially separated, backward-travelling waves generated at the more apical location (the 2F1–F2 site) must travel further to reach the ear canal than the waves generated at the basal location (the F2 site). Subsequently, waves from the apical site should be delayed relative to the basal site. They found that short latency peaks had the greatest amplitudes at higher levels, and longer latency peaks

are largest at low levels relative to high stimulus levels. The short latency peaks had a higher threshold, with rapid growth and little or no saturation. Later occurring peaks were present with lower level stimulation but amplitude growth is more gradual and perhaps saturates at higher levels of stimulation. These results are consistent with the idea that there are at least two sources that contribute to the generation of DPOAE. Shera and Guinan (1999) proposed that the fundamental distinction between the two sources is not only spatial location, but also source mechanisms. Kalluri and Shera (2000) tested the key predictions of the 'two-mechanism model' by separating the two components via selective suppression and spectral smoothing.

Kemp (1986) first coined the terms "wave-fixed" and "place-fixed". Theoretically, in a structure like the organ of the Corti loss of travelling wave energy through viscous forces is inevitable. Kemp (1986) indicated that, in an attempt to provide cochlear amplification the OHC mechanisms act to reduce mechanical energy loss and damping. Cochlear amplification refers to the active transduction process to enhance sensitivity in a narrow frequency band conceptually associated with the tip of the tuning curve. By virtue of its electromotility, a sharp mechanical impulse (to achieve sharp tuning) from OHC on each cycle of excitatory displacement, may be sufficient to cancel some viscous losses and improve cochlear performance. This involves conversion of metabolic energy into vibratory energy and increase in vibration at the peak of the travelling wave, and amplification still occurs when the travelling wave energy flows out of the cochlear partition. The initiation of this retrograde energy transmission in the cochlea (necessary for OAE) implies some form of localized perturbation of the forward travelling wave that would occur spatially if the normal gradation of physical propagation characteristics were irregular. In this case, the fixed perturbation place would respond to different phases of the stimulus as its frequency is changed. This would result in emission latency twice that of the forward travelling wave up to the fixed perturbation place. This mechanism is called "place-fixed'. Additionally, mechanical nonlinearity might modify propagation conditions at the peak of response. In this case, the place of re-emission moves with the travelling wave as frequency is changed. There is little phase change and this mechanism is called "wave-fixed". Ren (2004), has questioned the need for a reverse travelling wave to generate OAE. This is on the basis of measurements of the latency or delay of distortion at the middle ear and the time of arrival of the stimuli at the F2 cochlear location. The measurements did not show the expected time delay consistent

with a reverse travelling wave, thus, he proposed that DP may propagate to middle ear by a fast pressure wave. Despite this finding, the reverse travelling wave remains the most accepted explanation of OAE characteristics as currently understood, and the question of alternative pressure wave is yet to be resolved.

It is now accepted that the DPOAE recorded in the ear canal is the vector sum of the amplitude and phase interactions of two components, which arise from two mechanisms; distortion and reflection. Shaffer *et al*. (2003) provides a review of DPOAE generation sources and mechanisms. For the present purposes, they are referred to as wave- and place-fixed according to the usage of Knight and Kemp (1999). Figure 2.3 shows a schematic diagram illustrating the mechanism of generation of 2F1–F2 DPOAE in the normal cochlea (Shera and Guinan, 1999). Distortion, leading to wave-fixed components arises near the overlap region of the F1 envelope and the peak of the F2 travelling wave. These waves then propagate forward to their characteristic frequency place, where they are slowed by the mechanics of the basilar membrane, causing the delay typical of reflection emissions. Some energy is emitted back via a reverse travelling wave to the base of the cochlea and emitted into the ear canal, in addition to a number of reflection sites at the characteristic DP place and any imperfections basal to it. These reflections together constitute the place-fixed components. The wave- and place-fixed components combine to form the composite DPOAE in the ear canal.

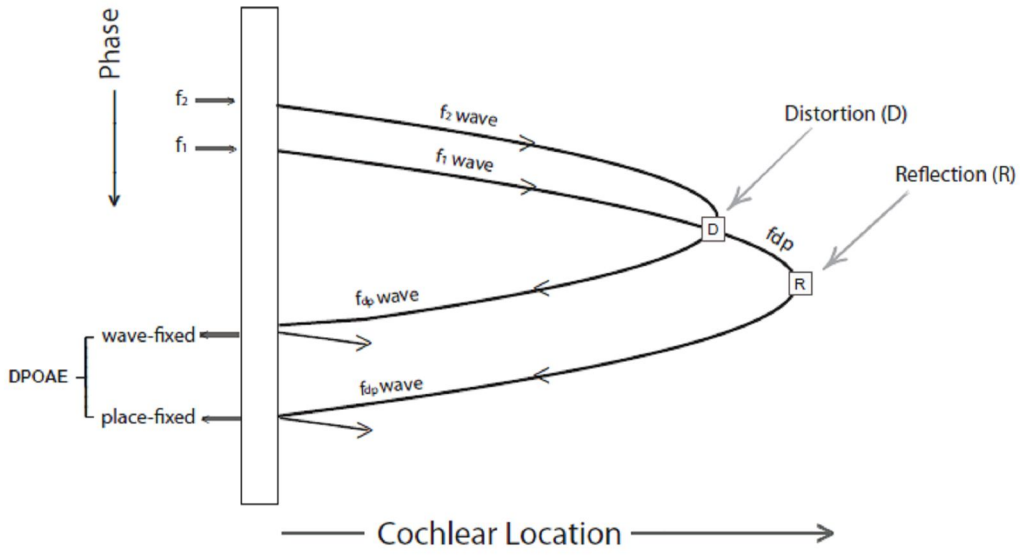

Figure 2.3. Schematic illustration of generation mechanism of 2F1–F2 DPOAE. (adapted with permission from Shera and Guinan, 1999).

The general assumption with a wave-fixed mechanism is that the emission is generated by distortion at a site that is an integral part of and moves smoothly with the stimulus travelling wave envelope in the cochlea as stimulus frequencies are swept, while the place-fixed component travels apically to its characteristic frequency place, where it may be reflected with a delayed latency (Shera and Guinan, 1999). For the 2F1–F2 distortion product, the wave-fixed component is considered to be generated close to the F2 place on the basilar membrane and reaches the ear canal via a travelling wave propagating in the reverse direction along the basilar membrane. Knight and Kemp (2000) proposed that in the case of the wave-fixed mechanism, the emission site is supposed to be an integral part of and to move smoothly with the stimulus travelling wave envelope as stimulus frequency is swept. Since the cochlear frequency scaling is approximately geometric, with the result that frequency shifts cause little change to the travelling wave shape, when a stimulus pattern is swept in frequency the phase at any point moving with the travelling wave envelope changes little. Therefore, any OAE contribution from that point would have a very shallow phase gradient. Distortion generated at the F2 place also propagates in the forward direction to the DP place, where it may be reflected. Zweig and Shera (1995) have proposed a series of reflecting or scattering sites existing along the basilar membrane and a mechanism of coherent reflection involving the sharply tuned basilar membrane excitation pattern. As stimuli are swept in frequency and their excitation patterns moves along the basilar membrane, the distortion product phase at the reflection site will change, thus increasing the OAE phase and creating a steep gradient.

DPOAE is a by-product of the outer hair cell mechanism. DPOAE are eliminated or reduced in amplitude in damaged cochleae due to noise exposure, ototoxic drugs and so on. Histological examination has revealed that outer hair cell damage is the anatomical correlate to the reduction of DPOAE amplitudes, thus verifying the involvement of outer hair cells in the generation of DPOAE (Brown, McDowell, and Forge, 1989). While it is well known that reduction in DPOAE amplitude provides an indication of functional or structural changes to cochlea, the relationship between DPOAE phase and cochlear functioning is not well understood. Previous studies suggest that the phase gradient against frequency, obtained using fixed frequency ratio sweeps is consistent with a combination of two different DPOAE emission components, as described in the

previous paragraph (Knight and Kemp, 1999, 2000, 2001). Steep and shallow phase gradients have been observed in the 2F1–F2 DP (Knight and Kemp, 1999) depending on whether a small or large frequency ratio is used. For a small frequency ratio, the phase gradient is steep, consistent with a predominantly place-fixed emission mechanism, while with a larger frequency ratio, the phase gradient becomes shallow and is more consistent with a wave-fixed mechanism. Knight and Kemp (2001) propose a model that suggests that the propagation of DP travelling waves is biased by the shapes of the primary travelling waves. For the more widely spaced primary frequencies commonly used to measure the 2F1–F2 DP, the reverse travelling wave in the F2 frequency region is promoted so that the wave-fixed component tends to dominate the response, thus explaining the shallow phase gradients observed with larger frequency ratios. The phase gradient technique has been used to study the changes in cochlear mechanisms due to external agents, such as, aspirin toxicity (Parazzinni *et al*., 2005a), and EMF radiations (Parazzinni *et al*., 2005b) and also to understand normal cochlear functioning (Wilson and Lutman, 2006). Interestingly, (Parazzinni *et al*., 2005a) found that the subtle changes in cochlear mechanisms (wave- and place-fixed) can be detected earlier by measuring phase gradient of DPOAE compared to amplitude of DPOAE or hearing thresholds. They reported that phase gradient increased by aspirin consumption, and did not recover even two days after cessation of aspirin intake, despite almost complete recovery of DPOAE amplitude and hearing threshold levels.

It is now widely accepted that the DPOAE recorded in the ear canal is a composite signal. The components of DPOAE can be separated at least by two methods; use of an ipsilateral suppressor tone (usually 15- 25 Hz) below the 2F1–F2 DPOAE (Heitmann *et al*., 1998) and by an inverse fast Fourier transform (IFFT)/time windowing method (Kalluri and Shera, 2001). Both of these methods have been found to give comparable results at moderate primary frequencies and for F2/F1=1.2 (Kalluri and Shera, 2001; Konrad-Martin *et al*.., 2001). The IFFT method was used in this thesis to separate DPOAE components because the suppression technique for component separation is difficult and more complicated to administer particularly with contralateral acoustic stimulation. Briefly, IFFT converts the DPOAE recorded at high resolution in the frequency domain into its equivalent in the time domain. The multiple peaks that appear in the time-domain represent DP with different time delays and hence from different generation mechanisms. Time-windowing is then applied to separate the amplitudes and

phases of the two components based on latency. After separation, the components are converted back to the frequency domain by using FFT.

The following sections provide critical review of most pertinent literature in three categories:

1. Changes in the auditory system due to mobile phone radiation.
2. Posture-induced changes in cochlear functioning measured by DPOAE.
3. DPOAE-based measures of efferent system functioning and the role of the efferent system in speech perception.

2.2. Mobile phones

Launched barely two decades ago, the mobile phone is the subject of intense research to ensure it poses no threat to human health. Mobile phone use and any consequent biological effects cannot be reduced to an issue of personal lifestyle, but involves the whole population, and should be considered as a high-priority environmental health concern. Mobile phone operations induce electromagnetic fields (EMF).The effects of EMF depend upon the frequency, which classifies into ionizing and non-ionizing radiations. EM radiation whose frequencies are greater than about 10^{16} Hz are called ionizing, while the frequencies less than 10^{16} Hz are termed non-ionizing radiations. Ionizing radiation can remove an electron from an atom to form an ion, and thus possesses sufficient energy to break the cell nucleus and can potentially dangerous to cause DNA mutations. Examples of ionizing radiations are X-rays, nuclear accidents. In contrast, non-ionizing radiations cannot cause DNA mutations directly. Mobile phones emit non-ionizing radiation. Mobile phone networks operate in one of three bands in Europe, 900 MHz, 1800 MHz and 2200 MHz, using two different technologies, Global System for Mobile Communications (GSM) and UMTS. GSM phones operate in the 900 MHz and 1800 MHz frequency bands and are commonly used in Europe, Africa and Asia in these bands. UMTS is the next generation (more popularly known as third generation '3G') of mobile phone technology, expected to result in widespread use of video phones and access to multimedia information at a cheaper price. UMTS phones operate approximately in the 2 GHz region.

The International Commission on Non-Ionizing Radiation Protection (ICNIRP) formulates and publishes exposure limit guidelines for EMF radiation, based on critical review of the published biological effects and health risks, and anatomic-mathematical models. Guidelines for EMF exposure limits relevant to mobile phones are expressed in terms of specific absorption rate (SAR). SAR is defined by the Institute of Electrical and Electronics Engineers (IEEE) as, *the time derivative of the incremental energy (dW) absorbed by (dissipated in) an incremental mass (dm) contained in a volume element (dV) of a given density.* SAR is defined by the ANSI standard as, *the time rate at which radio frequency electromagnetic energy is imparted to an element or mass of a biological body. SAR is expressed as energy flow (power) per unit of mass in units of W/kg.* When referring to human tissue, this means that the SAR is a measurement of the heat absorbed by the tissue. The SAR measurements can be stated mathematically as follows; $SAR = \sigma E^2/\rho$ (where, σ is electrical conductivity of tissue; E is internal electric field; ρ is mass density of tissue). In real-life, this means that a number of factors can determine the SAR. Most of them can be grouped as: (i) factors related to mobile telecoms transmitter devices, such as antenna, housing, internal design, etc.; (ii) factors related to head and position of the device such as, size and shape of head, hand for holding the set, spectacles, and other internal tissue parameters; and (iii) current distribution on the antenna and device, which would also be influenced by the head related variables. Theoretically and ideally, the SAR should be measured directly as a temperature increase in a localized area of tissue. To do this it would be necessary to insert calorimetric probes into a live mobile phone user's head in order to map SAR directly. However, this would be invasive and ethically unacceptable. As a result phantoms or model heads and mathematical simulations of exposed heads seem the only viable options for estimating SAR. However building a model head inherently involves approximations in tissue simulation and model complexity. Similar problems exist for computer models. Thus, there would be a considerable variation across different SAR estimations.

The SAR limit stated in international guidelines is 2.0 W/kg (ICNIRP, 1996, 1998). Individual national government agencies set SAR guidelines to indicate to the public the safe levels of electromagnetic exposure related to electrical appliances. The National Radiological Protection Board (NRPB), now a part of the Health Protection Agency (HPA) in the United Kingdom guideline initially recommended a limit of 10 W/kg in the head, which is much higher than the limit set by ICNIRP, but has now aligned with

ICNIRP. For employees, the HPA allows up to 10 W/kg at work. Calculations using mathematical models of the human head (phantom) have indicated that the current mobile phones comply with the ICNIRP limits (Hyland, 2000). Radio waves transmitted by the commonly used mobile phones in the UK are within SAR limits set by the ICNIRP, as all makes of modern GSM mobile phones, emit EMF radiation that results in less than 1 W/kg SAR in the head (Moulder *et al*., 1999). However, atypical antennas, operation conditions and heterogeneities of energy absorption inside the head might lead to higher localized SAR (Burkhardt *et al*., 1997; Dimbylow and Mann, 1994). It is important to note that the exposure limits refer to the maximum deposition of energy anywhere in the body. Normally, the maximum will be close to the surface and in the case of mobile phones in the region of application of the phone close to the pinna. The SAR at the inner ear will be substantially lower.

The following paragraphs provide a brief and general introduction to some of the objective tests that were used in the literature (and in Chapter 3) to determine the effects of EMF exposure on the human auditory system.

1. TEOAE are sounds recorded in normal cochlea in response to stimulation. However, to record TEOAE a normal middle ear is also required. TEOAE are also called click evoked OAE (CEOAE). TEOAE can also be recorded by tonal stimuli. The TEOAE responses are properties of normal ears and the prevalence is around 100%. The generation mechanism is not fully understood yet, but a number of studies have presented evidence supporting that the TEOAE are generated by a reflection of the travelling wave at micromechanical impedance perturbations in the organ of Corti (Kemp, 1980). TEOAE can also be recorded from other animal species, used in clinical research, such as mice, rats, guinea pigs, chinchillas, rabbits, dogs, and monkeys. Efferent auditory system functioning can be measures by OAE suppression. The most common and simplest suppression measurement is contralateral acoustic stimulation (CAS) of TEOAE. Several studies have found that TEOAE amplitude reduces by 1-3 dB with CAS (see Hall, 2000, for review).

2. DPOAE are generally recorded using two pure tone stimuli, close in frequency. The two stimuli are commonly called primaries (denoted by F1 and F2, with

F2>F1) and the corresponding sound levels are called L1 and L2 (usually, L1>L2). DPOAE can be evoked with F2/F1 ratio ranging from 1.1 to 1.3 (1.2 is commonly used). The most robust and mostly used DPOAE is the cubic difference distortion product denoted as the 2F1–F2 DPOAE. The results of DPOAE measurement are usually reported as a DP-gram; that is, amplitude (dB SPL) of DP plotted as function of either F2 or 2F1–F2.

The relation between stimulus intensity level and amplitude of DP is called DP growth or the input-output function (I/O). DP growth is usually recorded by systematically varying the level of primaries while the stimulus frequency and F2/F1 ratio is held constant. DPgowth is though to reflect compressive non-linear properties of the cochlea, and can be used to distinguish active and passive cochlear mechanics (Popelka *et al*, 1993; Withnell and Yates, 1998). The shape of the I/O function is variable across normal hearing individuals and even for stimuli at different frequencies for a given individual (Popelka *et al*, 1993; see Hall, 2000 for review).

3. Event related potentials (ERP) are brain responses that are evoked by sound processed in or near the auditory cortex (reviewed from McPherson, 1996). Following the presentation of an auditory stimulus, a pattern of neural activity occurs which can be detected remotely by electrodes positioned on the scalp. The far-field ERP recordings pick up the neural activity from the source of the potential by some scalp electrodes. The amplitude of such potentials are very low (in μV) and often more complex because the recorded response in practice is a combination of several responses that come from a large number of sources. Also, the nature of the potential detected at the scalp depends upon some crucial factor such as electrode placement and factors related to the physical properties of the volume conductor (e.g. tissue conductivity and orientation of group of neurons). ERP is usually recorded by presenting two stimuli in an odd-ball paradigm (i.e., one stimulus occurs more frequently than the other, to a predetermined criterion but in a random order). The resulting waveforms are called standard waveform (frequent stimuli) and deviant waveform (infrequent stimuli). ERP responses are characterized by a series of positive and negative components and are labelled according to their polarity and latency. The main

waves or peaks of standard waveform are P1, N1, P2 and occasionally N2. Similarly, the peaks of deviant waveform are N2 and P3, while other peaks can also be seen in varying degrees. P3 can be occasionally bimodal, having two components "a" and "b". P3 (or P300) occurs when a subject consciously recognizes the presence of a change in the acoustic stimulus and is elicited by task relevancy. The morphology and latency of the ERP components are highly dependent upon the evoking stimulus, acquisition parameters and participant. The latency regions in which P1, N1, P2, N2 and P3 can occur are 55- 80, 80-150, 145-180, 180-250 and 220-380 ms respectively (McPherson, 1996, pp. 9-10).

The critical analysis of literature on the potential effects of mobile phones EMF is categorically reviewed into (i) effects on the cochlea and (ii) effects on the auditory brainstem and central auditory nervous system. Table 2.1 presents the summary of the findings of some of the most pertinent published reports that have evaluated the effects of mobile phone EMF on the auditory system.

2.2.1. Effects on cochlear functioning

Cochlear functioning in mobile phone studies is measured by OAE in humans and laboratory animals, as well. As early as 1998, Grisanti and colleagues studied the effects of analogue cellular phones (total access communication system, TACS) on OAE in 25 normal hearing listeners. The EMF exposure considered both continuous and modulated signals. The frequency of exposure was (i) 900 MHz with 500 mW power for continuous signals, and (ii) the same signals modulated at 1 kHz – modulated exposure. No difference in TEOAE could be detected before and after exposure to continuous signals. In contrast, the distribution analysis using the mean and SD value of the distortion products in the subjects exposed or unexposed showed that the two distributions are clearly divided, indicating an effect induced by the radiation. Additionally, on an average, the DP growth function related to people irradiated with modulated microwaves was steeper than the same observed for non-irradiated people at all stimulus intensities. Although the DPOAE test protocol used in this study is unclear, the presence of a biological response using TACS cellular phones suggests the necessity of closely examining the studies in this field in order to verify if there may be some hazardous effects.

Marino *et al.* (2000) in a preliminary experiment evaluated the effects of microwaves (900 MHz) on cochlear receptors of eight Sprague-Dawley rats using DPOAE. The 10-week-old male rats were exposed to low intensity far-field (65 cm from the source) EMF with two input powers of 6 W and 30 W which corresponds to medium SAR of 0.2 W/kg and 1 W/kg respectively. The DPOAE were recorded at four primary tone combinations and compared before and after exposure. No statistically significant effect was obtained at either SAR value. The authors concluded that current results do not seem to point to a requirement for any more in-depth research into this specific aspect, however, future steps would be to analyze the prolongation of overall exposure time, in order to simulate a daily exposure and test of different exposure systems. The important limitations of this study are related to sample size, high variability found in sham exposure and absence of modulated signals. Moreover, the noise floors for the recorded DPOAE were not reported.

Ozturan *et al.* (2002) investigated the effect of EMF on human hearing in thirty normal hearing adults. TE- and DP-OAE were recorded before and after 10 minutes of EMF exposure. No measurable changes in OAE were detected. None of their subjects reported deterioration in hearing based on self-reports. The same research group (Kizilay *et al.*, 2003) studied the effects of chronic exposure to EMF on the hearing of adult and developing rats using DPOAE-gram and input/output functions after 1 hour of exposure each day for 30 days. The authors concluded that exposures of EMF from a mobile phone do not cause hearing deterioration at least at outer, middle and cochlear levels. Similarly, Moonerry *et al.* (2004) did not find any changes in TEOAE amplitudes following exposure to pulsed EMF in twenty normal hearing volunteers. However, these reports lack strong experimental designs such as no control condition and the exposure details were not reported.

Janssen *et al.* (2004) recorded DPOAE during exposure (i.e., between consecutive GSM signal pulses) and during sham exposure (no EMF) in 28 normally hearing subjects at frequencies around 4 kHz. GSM-like signals (900 MHz) were used with transmission pause increased from 4.034 ms (GSM standard) to 24.204 ms. Peak transmitter power was set to 20 W, corresponding to an average SAR of 0.1 W/kg. No significant change in the DPOAE level in response to the EMF exposure was found. However, when

undesired side effects (variation over time due to probe positioning) on DPOAE were compensated, in some subjects an extremely small EMF-exposure-correlated change in the DPOAE level (<1 dB) was observed. The authors state that, in view of the very large dynamic range of hearing in humans (120 dB), this observation is physiologically irrelevant. Moreover, the change was an increase in DPOAE amplitudes, which implies improved function. Also, the SAR level was very low at the level of cochlea.

Galloni *et al.* (2005a,b) did not find any changes in DP-gram and input/output functions of DPOAE in cochlear hair cell functionality of 58 Sprague-Dawley rats exposed to 900 MHz pulsed EMF in three different exposure protocols. The same group of authors as part of the EU-GUARD project studied the influence of EMF on the mechanism of generation of DPOAE (Parazzini *et al.*, 2005b). They concluded that effects of GSM exposure on the two DP components (i.e., wave- and place-fixed) and DP phase gradient was small and no statistically significant shift is evident after 10 minutes of exposure at the maximum power of a consumer mobile phone. Similarly, Uloziene *et al.* (2005) as part of the GUARD project concluded that 10 minutes of close exposure to EMF from a mobile phone has no immediate after-effects on hearing threshold level (HTL) measurements and TEOAE in young adult human subjects.

Paglialonga *et al*. (2007) in a double-blind design tested novel TEOAE measures (temporal and spectral fine structure) in 27 normal hearing subjects after exposure to EMF emitted by GSM phones. TEOAE data were analyzed both globally (broadband analysis) and using the Wavelet Transform (analysis of the time-frequency fine structure). There was no effect of exposure in either of the measures of TEOAE. The exposure system was same as (Parazzini *et al.*, 2005b).

Bamiou *et al*. (2008) in a double-blind design tested nine cases and 21 controls, who complained that they feel uncomfortable after prolonged mobile telephone use. Exposure duration was 30 min in pulsed, continuous RF emission or no emission test modes. The mean EMF output was delivered at a carrier frequency of 882 MHz and at SAR of 1.3 W/kg. They measured TEOAE in addition to vestibular tests. There were no significant changes TEOAE or vestibular system function due to exposure.

Table 2.1. Summary of some of the published reports on effects of mobile phone use on auditory system.

Study	N	Study design	Exposure details	Test parameters	Results	Comments
Grisanti *et al* (1998)	25	Pre- post with control	Pulsed & continuous (897.5 MHz; 500 mW), duration not reported	TEOAE, DPOAE growth functions	Affected in pulsed condition	DPOAE protocol is unclear
Kellenyi *et al.* (1999)	10	Pre-post-rest	GSM, 15 minutes	ABR	Delayed wave V	Normative data was not appropriate
Marino *et al.* (2000)	8	Pre-post with control	Continuous (900 MHz; SAR 0.2 & 1.0 W/kg), prolonged exposure	DPOAE	No effect	Animal model, Small sample size
Ozturan *et al* (2002)	30	Pre-post	GSM (900 MHz), the exact details were not reported; 10 minutes	TEOAE, DPOAE	No effect	Weak study design
Arai *et al* (2003)	30	Pre-post	Pulsed EM (800 MHz; 0.8 W), 30 minutes	ABR, AMLR	No effect	Did not analyze inter-peak latencies
Bak *et al* (2003)	45	Pre-post	EMF (450, 935 and 1800 MHz), 20 minutes	ABR	No effect	Interference was checked
Kizilay *et al* (2003)	14	Pre- post with control	GSM (900 MHz; SAR 0.95 W/kg), 1 hour for 30 days	DP gram, I/O functions	No effect	Animal model, High DP noise floor
Hamblin *et al* (2004)	12	Single-blind, crossover with sham	GSM (894.6 MHz; peak power 2 W), 1 hour	N1, P1, N2, P2, P3 and RT	Affected N1, P3 and RT	Small sample re: the high variability
Janssen *et al*. (2004)	28	Comparative, genuine and sham trials	GSM like signals (900 MHz; SAR 0.1 W/kg)	DPOAE amplitudes	No adverse effects	Increase in amplitude around 1 dB, novel
Monnery *et al* (2004)	12	Pre- post	Exposure details not reported	TEOAE	No effect	Weak study design
Galloni *et al* (2005)	58	Pre-post with sham	GSM, Long-term exposure in 3 different protocols	DP-gram, input/ output function	No effect	Animal model, limited frequency
Oysu *et al* (2005)	18	Pre-post	GSM (900 MHz; SAR 0.82 W/kg), 15 minutes	ABR	No effect on absolute & IPL	No control exposure
Parazzini *et al* (2005a)	15	Pre-post with sham, double-blind	GSM (900 MHz, power 2 W; 1800 MHz, power 1 W), 10 minutes	Place- & wave-fixed, DP phase	No effect	Novel DP measure
Uloziene *et al* (2005)	30	Pre-post with sham	Same as in Parazzini *et al* (2005a)	PTA & TEOAE	No effect on amplitudes	Limited due to TEOAE bandwidth
Stefanics *et al*. (2007)	30	Pre-post with sham, double blind	Same as in Parazzini *et al* (2005a)	ABR	No effects	
Paglialonga *et al*. (2007)	27	Pre-post with sham, double blind	Same as in Parazzini *et al* (2005a)	TEOAE	No effects	Novel; spectral and temporal fine structure.

The literature review about the possible influences of EMF from mobile phones on hearing using OAE in humans and animal models suggest the results are not always consistent. The inconsistencies may have been due to a number of reasons, such as brief exposure duration in humans, low and variable power output of the phone, study designs without sham or control condition and poor OAE protocols. In animal models the measurements had been restricted by the frequency spectrum of the DPOAE instrument designed for human use. Higher frequency measurements (particularly, UHF-DPOAE) could be able to reveal more comprehensive information about the effects of mobile phone EMF exposure. Some of the important limitations of most of the studies reported are related to inadequacies in sample size calculation, and lack of description of the exposure system.

2.2.2. Effects on auditory brainstem and central auditory system

Kellenyi *et al.* (1999) studied the effects of GSM phones on auditory brainstem response (ABR) in 10 normal hearing healthy adults (mean age: 29.3; SD: 8 years). The activation of the mobile phone was software controlled and blind to the subjects, who were exposed to 15 minutes of pulsed EMF. ABR was recorded in the EMF-exposed right ears and non-exposed left ears using clicks at a rate of 27 Hz at 80 dB. They found that the wave V peak latency in the exposed side was significantly delayed by 0.207 ms compared to the baseline latency. On the non-exposed side, a latency shift of 0.029 ms was observed which was interpreted by the authors as a contralateral crossed interference effect. They performed extended pure tone audiometry (150 Hz to 10 kHz) in three subjects to explain the observed latency shift. They found 15-18 dB change on the exposed side only, however, none of the subjects complained of a hearing loss. They explain that high frequency hearing loss was due to thermal effects and ionic membrane shifts caused by pulsed EMF emitted by the mobile phone and that the changes were similar to the hearing damage after noise exposure. Their sample size was small (n=10) and subject selection was not adequately controlled. The normative data on ABR (Pytel *et al.*, 1986) with which the results are compared appears to be old and used a different recording instrument. Even then, the observed effect (0.207 ms) was smaller than the SD ± 0.39 (mean of 5.63) yet was interpreted as significant. Such small latency changes could also be due to the normal body temperature variations without any functional relevance. Surprisingly, the authors did not reveal any follow up measures, such as, if the subjects

regained their normal latency and if so, after what duration. Importantly, if some changes do occur in the ABR, it would be a serious concern.

Jech *et al.* (2001) measured event related potentials (ERP) in a visual odd-ball task to examine the effects of a 900 MHz GSM phone on brain activity and reaction time (RT). Their sample consisted of 17 patients diagnosed with narcolepsy-cataplexy. ERP testing began after 5 minutes of exposure to a mobile phone set to continuously transmit at a maximum power output of 2 W over the right hemisphere. Results revealed decreased N200 amplitude, increased P300 amplitude and a shortened RT to target stimuli during genuine exposure relative to sham exposure. No effect on the latencies of endogenous components was found. However, it is difficult to know how representative these results are due to the specific nature of the population tested.

Arai *et al.* (2003) investigated if high-frequency pulsed EMF (800 MHz; 0.8 W) emitted by a mobile phone has short-term adverse effects on the human central auditory system. They analyzed ABR, ABR recovery function (V peak) and auditory middle latency responses (AMLR) at 80 dB peak equivalent SPL (peSPL) in 15 normal hearing volunteers before and after using a mobile phone for 15 minutes. They failed to detect any short term effects on the ABR or AMLR parameters and recommended for the need of more follow up studies to evaluate long-term effects of mobile phone use and to investigate possible changes in auditory function more exclusively (e.g., using both behavioural sensitivity, discrimination measures and electrophysiological measures). The authors, however, did not analyze the inter-peak latencies, particularly (I-V), which is known to be the most sensitive ABR parameter to indicate any subtle dysfunction at the level of brainstem.

Bak *et al.* (2003) evaluated the effects of EMF (frequencies; 450, 935 and 1800 MHz) on ABR in 45 young healthy volunteers during and after repeated phone activation for 20 minutes. Prior test calibration on a phantom did not show the influence of the external EMF generated by the mobile phone on the ABR recording instrument. For neither EMF frequency were differences observed in absolute wave and inter-wave latencies compared to the baseline ABR pattern. They conclude that commonly used mobile phones do not affect propagation of electrical stimuli along the auditory nerve to auditory brainstem.

Hamblin *et al.* (2004) explored the sensitivity of auditory evoked potentials (AEP) to electromagnetic transmissions (frequency, 894.6 MHz; power output 2 W). Twelve normal hearing participants, aged 19-44 years attended two sessions (genuine and sham) one week apart. AEPs were recorded in an odd-ball paradigm. N1 and P2 were analyzed for non-target waveforms, and N200 and P300 were analyzed for target waveforms. They found that in genuine relative to sham exposure N1 amplitude and latency were reduced, with reduction larger over midline and right hemisphere sites. P3 latency was delayed in the genuine exposure condition; however, as this was greatest at left frontal and left central sites the interpretation of this result is unclear. RT was also increased in the genuine condition. No difference in accuracy of the task was found. The results suggest that EMF may affect neural activity; however, caution should be applied due to small sample size. An important interpretation based on these results could be that mobile phone EMF leads to an increased speed of stimulus processing but a decreased capacity to deal effectively with this information. The authors assert that due to the fact that typical mobile phone users usually experience lower power intensities and shorter periods than those employed in the study, implications for normal mobile phone use are limited.

Oysu *et al.* (2005) evaluated the influence of EMF in 18 normal hearing adults. Mobile phones emitting signals in the region of 900 MHz and the highest SAR of 0.82 W/kg were positioned in direct contact with the right ear for 15 minutes. The differences in the mean latencies of waves I, III and IV were not significant in initial and post-exposure ABR measurements at both 60 and 80 dB nHL levels. Similarly, differences of the mean inter-peak latencies were not significant. They conclude that acute exposures to mobile phone EMF do not cause perturbations in ABR. However, they contended that these negative results should not encourage excessive mobile phone communications, because minor biological and neurophysiologic influences may not be detectable by the current technology.

Sievert *et al.* (2005) used ABR to evaluate the effects of EMF in 12 normal hearing adults. ABR was recorded (50, 55, 60 dB pe SPL) before, during and after exposure to standard mobile phones (frequency 889.6 MHz; SAR at 18 mm deep 1.93 mW/g) in both continuous and pulse modes. No impact on ABR in terms of absolute and inter-peak latencies could be found, and hence they concluded that there is no short-term effects on the auditory system. However they cautioned that any long-term effects cannot be excluded by this study.

Oktay and Dasdag (2006) in a between subject design evaluated the long-term effects of mobile phone use on hearing. The three groups of subjects were (i) 20 frequent users (approximately 2 h per day for four years) (ii) 20 occasional users (10-20 min per day for four years), and (iii) 20 non-users (control group). No differences were observed between infrequent mobile phone users and control group, but pure-tone thresholds in frequent users were found to be higher than those in either infrequent users or control subjects at 4000 Hz for both bone and air conduction for right ears, and at 500 Hz and 4000 Hz bone and air conduction for left ears. Inter-peak latencies (I-III, III-V, and I-V) were not different among the groups ($p \geq 0.05$). It is not clear why the change in auditory thresholds was seen but not in ABR; moreover, the other confounding factors were not controlled. Stefanics *et* al. (2007) in a double-blind study did not find any changes in the ABR in 30 normal hearing adults due to 10 minutes of exposure to GSM mobile phones.

Critical analysis of literature reveals possible sources of inconsistencies in results of various studies. This is mainly related to study design and experimental instrumentation. A possible limitation could be due to the electromagnetic interference between the EMF generating system and the AEP recording instrument, particularly the electrodes while studying during exposure effects. Only two studies have addressed these issues (Bak *et al.*, 2003; Hamblin *et al.* 2004). Most of the studies did not perform a sample power calculation to determine the appropriate sample size, which would have provided meaningful differences and accurate interpretation of results. This is particularly important due to the variations in AEP parameters. Some studies provide no information about the nature of EMF source. Additionally, only one study considered a stringent design of considering sham exposure (Hamblin *et al.*, 2004). Also, exposure levels in some of the studies may have been too low at the level of inner ear and none of the reports considered a double-blind design to avoid both tester- and subject-related biases.

An important observation from the review of literature is that, to date no studies have reported the effects of mobile phone exposure on the auditory efferent system. The efferent system modulates cochlear functioning.

2.3. Posture-induced changes in cochlear functioning measured by DPOAE

Changing body position from sitting to supine induces an increase in the intracranial pressure (ICP) of the cerebrospinal fluid (CSF) due to gravity (Davson, 1967; Chapman, 1990). Auditory functions such as auditory thresholds (Corso, 1962 and Macrae, 1972), sound localization (Lackner, 1974) and auditory threshold microstructure (Horst *et al*., 1983) have been reported to change with posture. Studies by Marchbanks (1982) reported that middle ear admittance also changes with body position. Effects of body position have been investigated on stimulus frequency emissions (Wilson, 1980), spontaneous OAE (Wilson and Sutton, 1981) and transient-evoked OAE (Antonelli and Grandori, 1991; Fukai *et al*., 2005), and DPOAE (Büki *et al*., 1996, 2000). Such changes originate either from middle ear and/or cochlea in relation to body position or CSF pressure. These studies indicate that posture-dependent changes of ICP induce variations of intra-labyrinthine pressure and thus cochlear functioning.

There appears to be general consensus that there is a correlation between ICP and intra-cochlear pressure, accounted for by anatomical connection between CSF and cochlear fluid systems through the cochlear aqueduct (Carlborg *et al*, 1982). Increased ICP, induced by changes in body position would therefore result in subsequent increase in intra-cochlear pressure (Magnaes, 1976; Parsons and Wilson, 1983). Two mechanisms have been suggested to contribute to changes in cochlear function due to increased ICP; the modified intra-cochlear pressure may alter cochlear responses by acting directly on the structures of the cochlea (e.g., the hair cells) or the modified intra-cochlear pressure may increase the stiffness of the middle ear system (e.g., annular ligament that attaches the stapes of the middle ear cavity to the oval window of the inner ear). Böhmer (1993) showed that changes in intra-cochlear pressure have little effect on cochlear function; however, it is well documented that increases in the stiffness of the annular ligament substantially reduce middle ear sound transmission at frequencies below middle ear resonance frequencies (Büki *et al*., 1996; Lynch *et al*., 1982; Merchant *et al*., 1996). Increase in ICP due to manipulation of body position, in principle, should be evident in OAE.

Frank *et al*. (2000) compared the changes in amplitudes of spontaneous otoacoustic emissions (SOAE), TEOAE and DPOAE due to changes of ICP in 12 normal hearing adults and in 5 patients with hydrocephalous undergoing intraventricular pressure

monitoring. OAE were recorded in two body postures: horizontal (–30º) relative to supine position. In the normal hearing group, an increase of ICP led to decrease in the amplitudes of SOAE by 3.3 dB and TEOAE by 2.1 dB, while the amplitudes of DPOAE showed a frequency dependent effect with maximum reduction of 7.9 dB at 1 kHz (F2 frequency). The amplitude of DPOAE decreased by 2 dB at low frequencies corresponding to an ICP increase of 19.2 cm H_2O in the patient group, indicating the suitability of DPOAE for non-invasive monitoring of ICP changes in patient population.

Büki *et al*. (2000) showed qualitative changes in amplitude and phase of DPOAE due to changes in ICP in adults with normal hearing and in 5 hydrocephalous patients. The phase changes are largest at frequencies below 2 kHz. However, the DPOAE measurements show substantial inter-subject variability and they did not control for the parameters of middle-ear pressure and intra-subject variations in DPOAE.

In order to examine the potential of DPOAE for non-invasive monitoring of ICP, Voss *et al*. (2006) measured posture induced changes in DPOAE in seven normal-hearing subjects at four postures (90°, 0°, –30°, and –45° to the horizontal), with estimated ICP changes from 0 to 22 mm Hg. DPOAE were measured for F2 frequencies from 750 to 4000, with F2/F1 ratio of 1.2 and L1= 65 dB and L2 = 55 dB. At F2 frequencies below 1.5 kHz, DPOAE magnitudes significantly reduced as posture changed from 90º to –45º, with minimal differences above 1.5 kHz. The tympanometric measurements were conducted to monitor the middle ear status although at low resolution (±50 daPa).

2.4. Efferent auditory system functioning and DPOAE-based measurement

Olivocochlear bundle (OCB) neurons form the auditory efferent system that originates in the auditory brain stem and terminates in the organ of Corti, thereby allowing the central auditory nervous system to influence the function of cochlea, mainly OHC mechanisms. Initially, the auditory efferent pathway was classified into ipsilateral and contralateral systems following the pioneering work of Rasmussen (1946). A revolution in our knowledge of OCB was the re-classification of this system into MOCB and LOCB (Warr and Guinan, 1979). It was then recognised that all the earlier experiments on efferent effects and all efferent recordings appeared to be from the MOCB. MOCB are thick and

myelinated fibres that originate from the medial part of the superior olivary complex. These fibres innervate the OHC directly.

The turning point in studying OCB in humans was brought about by the (i) discovery of otoacoustic emissions, and (ii) knowledge that the MOCB responds to ipsilateral and contralateral sound with feedback sharply tuned to a cochlear location corresponding to the same frequency as the efferent fibre (Robertson, 1984). Over the years, contralateral acoustic stimulation has been used to evoke MOCB activity and investigate its effects on the auditory compound action potential (N1), single auditory nerve recordings and OAE (Buño, 1978; Collet *et al*., 1990; Warren and Liberman, 1989). Since recordings of OAE are non-invasive they can be applied in human listeners with normal auditory function to measure the OCB effects. Additionally, such studies may aid exploring the functional role of the efferent auditory system.

The amplitude of OAE recorded from one ear can be changed by presenting sounds to the same, opposite or both ears. This change in amplitude is called OAE suppression, because more commonly OAE amplitudes are reduced (Berlin *et al*., 1993; Collet *et al*., 1990; Moulin *et al*., 1993; Mott *et al*., 1989). Depending upon the ear of stimulation (same ear, opposite ear or both ears) this effect is called ipsilateral, contralateral or binaural suppression respectively. This sound-induced reduction is a normal phenomenon mediated by the efferent auditory system. This is called the medial olivocochlear (MOC) reflex as it is thought to be more directly related to the functioning of medial efferent system (see Guinan, 2006, for review). In principle, the suppression can be recorded via all types of OAE, but each has its advantage and disadvantages. The most common and simplest way of measurement is contralateral suppression of TEOAE. Literature on acoustic suppression of TEOAE is quite extensive.

A large body of neurophysiological literature suggests that the MOC system plays a critical role in OAE suppression as it attenuates the cochlear response to sound by reducing the gain of the OHC mechanical response to stimulation (Galambos, 1956; Murugasu and Russell, 1996; Wiederhold, 1970). Several experiments done in animals using electrical stimulation of MOB confirm that these effects are due to the efferent system and are mediated by the neurotransmitter acetylcholine (ACh) (Guinan, 2006 for review). Hence, the suppression is attributed to the MOC system and is considered as the

strength of MOC system/reflex (Collet *et al*., 1990; Maison and Liberman, 2000). CAS has received increasing interest and has opened the possibility of evaluating the physiology and patho-physiology of the MOC in clinical conditions (for review, see Hood, 2007).

Most of the research suggests that the change in DPOAE amplitude due to CAS is around 1 dB or less, and this change depends on primary tone levels, level of CAS, type of CAS (noise or tone), and frequency range (Bassim *et al*., 2003; Chery-Croze *et al*., 1993; Di Girolamo *et al*., 2001; Giraud *et al*., 1997b; James *et al*., 2002; Janssen *et al*., 2003; Kim *et al*., 2002; Lisowska *et al*., 2002; Moulin *et al*., 1993; Müller *et al*., 2005; Moulin and Carrier, 1998; Sliwinska-Kowalska and Kotylo, 2002; Timpe-Syverson and Decker, 1999; Williams and Brown, 1995; Zhang, Boettcher and Sun, 2007). In general, there is a considerable amount of variability in MOC effects when measured by DPOAE. Williams and Brown (1997) and Müller *et al* (2005) in humans, and Kujawa and Liberman (2001) in animals found the evidence of CAS induced bipolar changes in DPOAE (transition from enhancement to suppression). Maison and Liberman (2000), who used a matrix of 176 different primary tone level combinations, found that variation of the primary tone level by only 1 dB could result in changes of ipsilateral adaptation of DPOAE of more than 30 dB, including a change in sign of the amplitude change (bipolar effect). Recently, Wagner *et al*. (2007) in a carefully designed study evaluated the dependence of MOC effects on the fine structure of the DPOAE. They found that MOC effects depend upon the peak or notch of the fine structure and are critically related to the primary tone levels. On average, MOC effects were of the order of 2-3 dB at frequencies with distinct fine structure dips.

Compared to the literature on DPOAE amplitudes, studies on effects of CAS on DPOAE latency or phase are very limited and provide conflicting results. Giraud *et al*. (1997b) found that effect of CAS on DPOAE latency in normal hearing individuals was dependent upon the frequency and the latency was shortened at low frequencies (0.8-2.3 kHz). In the vestibular neurotomized patients (presumably no effective MOC system) the results were variable and depended on the nature of the pathology and surgery. In a related study Büki, Wit and Avan (2000) used phase of DPOAE to separate the effects of MOC and middle ear muscle reflex, and found approximately 10–15^0 of phase shift at 0.5-3 kHz with CAS. On similar lines, Sun (2008) showed a minimum DPOAE phase

change at low CAS level, while high level CAS caused a substantial phase lead for 1 and 2 kHz and with increasing frequency, phase lag became more notable. In contrast, Williams and Brown (1997) in 4 normal hearing participants did not find any effect of CAS on mean group delay of the DP. In 15 children (11-13 years) Silva and Ysunza (1998) also did not fInd any effect of CAS on latency of DPOAE. Similarly, Relkin *et al*. (2005) reported minimal changes in phase of DPOAE in rats due to CAS.

Despite the anatomy of OCB being well defined, it still remains unknown what is the function of these fibres, approximately 1400. Although there is little evidence existing on the degree which they function, there are several speculative roles of the MOCB in hearing: (i) anti-masking effects modulating the feedback control of the auditory periphery (Nieder and Nieder, 1970), subsequently supported by a large body of physiological studies which activated the efferents either through electrical stimulation or contralateral noise (Dolan and Nuttall, 1988; Guinan and Gifford, 1988; Kawase *et al*., 1993; Kawase and Liberman, 1993; Winslow and Sachs, 1988); (ii) protection of the auditory system from acoustic injury (Cody and Johnstone, 1982; Rajan, 1990); (iii) selective attention (Scharf *et al*., 1994, 1997); (iv) auditory imprinting and auditory development (Walsh *et al*., 1998). Among these hypotheses, the anti-masking effect has received the most extensive investigation, and perhaps the strongest experimental evaluation.

Behavioral studies of the efferent anti-masking function are not very common in contrast to the large body of physiological literature on anti-masking functions of the MOCB. Moreover, most of the behavioral studies have used non-speech sounds. For example, Micheyl and Collet (1996) found a possible relationship between detection of tones in noise and the strength of efferent activation, as measured by contralateral suppression of otoacoustic emissions. In contrast, Scharf *et al*. (1994, 1997) conducted extensive behavioral studies in listeners with vestibular neurectomy, a surgical procedure that severs both the vestibular nerve and the efferent nerve to alleviate vertigo. They systematically measured the effects of neurectomy on detection and discrimination of tones in noise. They found essentially no difference between the operated ear and the non-operated ear in detection of tones, intensity discrimination, frequency selectivity, loudness adaptation, frequency discrimination within a tonal series and in-head lateralization. The only evidence they found is that the lack of OCB input impairs the

ability to focus attention in the frequency domain. Nevertheless, to interpret the results it is necessary to understand that all the participants had one good ear. Although the role of the OCB in selective attention is still controversial, Scharf *et al*. (1997) could replicate these basic findings in 16 case studies and contend that conceivably, a strong efferent effect becomes apparent only for complex patterns of sound. Although less studied and equivocal, such a possibility receives some support from poorer vowel discrimination in monkeys after sectioning the OCB (Dewson, 1968). Only four studies with speech stimuli in humans have been reported. Giraud *et al*. (1997a) reported a negative correlation between the improvement of speech-in-noise intelligibility induced by contralateral noise and strength of the olivocochlear feedback. Zeng *et al*. (2000) found little effect of vestibular neurectomy on pure-tone detection and discrimination in quiet. Nevertheless, they noted efferent section increased loudness sensation (one participant), reduced overshoot effects (five participants), accentuated "the midlevel hump" in forward masking (two participants), and worsened intensity discrimination in noise (four participants). Poorer speech in noise recognition was also reported in the operated ear than the non-operated ear in three out of four participants tested, but this finding was confounded by the hearing impairment. Similarly, Kumar and Vanaja (2004) reported correlation between suppression of emissions and speech identification scores at +10 and +15 dB signal-to-noise ratio (SNR) in ten children with normal hearing. However, Harkrider and Smith (2005) rejected the hypothesis that individual differences in efferent activity of the MOCB contribute to the inter-subject variability in the amount of background noise accepted while listening to monotic or dichotic speech or the inter-subject variability in speech recognition in monotic noise. Recently, Wagner *et al*. (2007) reported that speech reception thresholds in noise do not correlate with efferent olivocochlear reflex (measured via DPOAE) in humans with normal hearing. This lack of correlation may be due to the nature of the speech perception task (a very basic threshold task) in their study.

2.5. Statement of the problem

The goal of this thesis was to investigate subtle changes in cochlear mechanisms and functioning of the efferent auditory system via traditional and mechanism-based OAE measures. A secondary aim was to substantiate the functional relevance of the efferent auditory system in speech perception, in order to asses the clinical significance of suppression measurements. Potential changes in cochlear (and auditory) functioning were induced by mobile phone radiation exposure and change in body position. While body position induces changes in cochlear function the potential effect of mobile phone exposure was unknown. Specifically, this thesis tested three independent, yet closely related hypotheses: (i) exposure to mobile phone radiation induces changes in auditory functioning, especially cochlear functioning (tested in Chapter 3), (ii) changes in cochlear functioning (posture-induced) and contralateral acoustic stimulation have differential effects on wave- and place-fixed components of DPOAE (tested in Chapter 4 and 5, respectively), and (iii) the efferent auditory system plays an anti-masking role in speech-in-noise perception (tested in Chapter 6). The first hypothesis was tested via a set of audiological tests including traditional OAE measures: TEOAE, contralateral suppression of TEOAE, DP-gram and DP growth. In contrast, the second hypothesis was tested using novel (wave- and place-fixed) DPOAE measures. Finally, the third hypothesis was evaluated using a combination of psychophysical and physiologic methods. While each of these experiments is self-contained, collectively they represent a more comprehensive measurement of the peripheral auditory system, especially cochlear and efferent mechanisms.

CHAPTER 3

CHANGES IN AUDITORY FUNCTION DUE TO EMF EXPOSURE

3.1. Overview

The objective of this experiment was to investigate the potential changes in auditory function (with main focus on the cochlea and efferent auditory system) following acute exposure to electromagnetic fields (EMF) emitted by UMTS phones on the auditory system. The scientific and general rationales for this investigation could be derived from the following observations and assumptions;

(i) Microwave hearing (i.e., auditory stimulation by non-auditory stimuli, e.g., thermal stimuli),
(ii) Vulnerability of the auditory system (particularly, OHC),
(iii) Position and use of the mobile phones, and
(iv) Miscellaneous factors.

(i) Microwave hearing: The theoretical basis that could support a hypothetical effect of EMF specifically on OHC could be derived from the phenomenon of microwave hearing in humans and animals, and from the following fundamental studies using animal models. The observation that pulse RF fields could induce an auditory effect, i.e., an auditory sensation, both in humans (Frey, 1962; Frey and Messenger, 1973) and in animals (Frey 1967; Guy *et al.*, 1975; Lebovitz and Seaman, 1977; Taylor and Ashleman, 1974) raised the idea to investigate this microwave hearing phenomenon. The most commonly accepted hypothesis for this is the thermo-elastic expansion of the soft tissues inside the head (Foster and Fynch, 1974; Guy *et al.*, 1975). The absorbed energy produces small but fast changes in temperature which induces a thermo-elastic wave, which is transmitted through the temporal bone to the inner ear where the receptors respond to it normally as when stimulated acoustically.

Lebovitz and Seaman (1977), and Seaman and Lebovitz (1987, 1989) performed a series of studies to analyze the response of single auditory neuronal units in cats following exposure to pulsed RF. One of the important findings was the similarity in response properties (of the auditory nerve and cochlear nucleus neurons) due to microwave

heating and acoustic clicks. This confirms that microwave hearing is due to alterations in inner ear and/or auditory nerve activity rather than more central contributions. Another observation was the response of the neurons to microwave heating is non-linear and frequency-dependent. The SAR thresholds to evoke a response in cochlear units are generally greater than 6 W/kg and depend upon the characteristic frequency of the unit.

This microwave hearing serves as the basis to define the guidelines for the human exposures to pulsed RF. The first study for these purposes uses computational head models with a homogenous spatial distribution of SAR inside the head (Olsen and Lin, 1981). However, in fact, the head tissues would greatly influence the SAR distribution and possibly the propagation of thermo-elastic waves. Watanbe *et al.* (2000) conducted a study based on the exposure to pulse microwave using an anatomic head model and improved method to calculate the EMF distribution and thus to solve the thermo-elastic wave. The results are explained in terms of pressure waves at the cochlear level and head. They concluded that it would be necessary to have a power density 300 times greater than the ones used to reach the perception threshold level typical of microwave hearing (Lin, 1980).

(ii) Vulnerability of the auditory system: OHC enhance the auditory sensitivity and frequency selectivity by amplifying low-level sound signals mechanically (Dallos, 1992). *In vitro*, OHC are capable of fast contractions and elongations of their cell body in response to an electric field (Brownell *et al*., 1985). This electromotility is suggested to result from a protein in the OHC baso-lateral membrane that undergoes structural re-arrangements in response to changes in the trans-membrane voltage, and is assumed to produce the amplification of vibrations in the cochlea during acoustic stimulation (Zheng *et al*.., 2000).

Several studies in animals and humans have consistently indicated and it has been now concluded that the auditory system, particularly OHC, are susceptible to a number of external agents such as, noise, ototoxic drugs, virus, systemic diseases and even music. Moreover, the OHC dysfunction can be evaluated and monitored using OAE before it is evident behaviourally (for review, Hall, 2000). Given the phenomenon of microwave hearing and the vulnerability of OHC, there could be a potential interaction between mobile phone EMF and OHC.

(iii) Position and use of mobile phones: The most common mobile phone use necessitates holding the handset in closet proximity to the ear, thereby, producing localized SAR over the area, and possibly leading to high energy deposition in the cochlea. The cochlea possesses micro-homeostatic mechanisms that are essential for mechano-electrical transduction of the OHCs, and disturbances to these may emerge due to high-energy absorption in the cochlea. In addition, there are at least two dozen of biochemical substances found in cochlear and efferent structures (refer Hall, 2000; pp.54, for a complete list) , a few, (or some) of these might react in a different way due to high local energy leading to OHC dysfunction.

In general, the use of mobile phones is so widespread that estimates indicate about 2 billion users by 2007 (http://www.geekzone.co.nz, accessed December 2005), far exceeding the telephone use via landlines. The newer 3G-phone users top of 5 million (http://www.3gnewsroom.com, accessed December 2005). Davidson and Lutman (2007) have found extremely high prevalence of mobile phone usage among a student population in the UK. Considering these high figures of mobile phone users a small elevated risk (if any) could raise serious public health concerns globally. Adding to this, there could be individual differences in susceptibility, for example, because the head and auditory system are still developing in the teenagers, children might be more vulnerable than adults. Similarly, heavy users, and users with existing ear disorders might be at high risk.

(iv) Miscellaneous considerations: The research on effects of GSM phones on the auditory system has been established in recent years. A review of the literature has been described in an earlier chapter (Chapter 2). To date there have been no reports on the effect of UMTS on the auditory system. It has been known mathematically that a small change in frequency and modulation (as in UMTS phones) would influence the EMF and possibly change its effects. An expected benefit of such investigation would help in establishing measures of prevention of the auditory effects (if any). Much technological advancement can be anticipated in future, which would perhaps require many more wireless systems to be coupled to the ear. Moreover, the level of background EMF is set to increase with developments in wireless communications and data networks.

3.2. Method

3.2.1. Participants

Participants were healthy young adults without any evidence of hearing or ear disorder, corresponding to the ISO definition of *otologically normal*. The idea was to test a group that is representative of the population of young otologically normal people. Absence of pre-existing hearing or ear disorder will maximise the sensitivity of the study to detect small changes that might occur. Specifically, participants satisfied the following criteria:

- Age between 18 and 30 years.
- In a good state of general health.
- Hearing threshold levels (HTL) in both ears no worse than 20 dB at any of the standard audiometric frequencies between 0.5 and 8 kHz.
- No evidence of conductive hearing loss based on air-conduction and bone conduction audiograms.
- Normal tympanograms and acoustic reflexes present in both ears for stimulation using a 1-kHz tone at 100 dB HL.
- Normal appearance of the tympanic membrane on otoscopy.
- No history of otological disorder.
- No history of familial hearing disorder.
- Noise exposure infrequent (e.g. night clubs) and without persistent effects.
- No self-reported hearing difficulty or persistent tinnitus.
- No exposure to ototoxic drugs by injection or topical spray (e.g. for severe burns).
- No excess consumption of alcohol or drugs during 24 hours prior to testing.

Acceptance as participants was based on otoscopy, audiometry by air conduction (0.5, 1, 2, 3, 4, 6, 8 kHz) and bone conduction (0.5, 1, 2 kHz), tympanometry and acoustic reflex testing, and a simple screening questionnaire[1] concerning medical and otological history (see Appendix 3.1) filled in by the subject in the presence of the investigator. Additionally, participants were excluded if (i) there was excessive wax in the ear canals. (This might interfere with the ability to complete screening tests and might possibly preclude accurate OAE measurement), (ii) there was any other contraindication or

[1] This form was also used in other experiments.

features that might affect performance on the tests (e.g. large ear rings or studs that could not be removed conveniently), or (iii) if they did not complete the entire testing.

The sample size was calculated based on the normative data on TEOAE (SD=2.3 dB) from pilot studies conducted at various labs within the EMFnEAR consortium and from relevant literature (Hall, 2000). The assumptions for the calculation were to use a one-tailed Student's t test for related samples with significance at $p<0.05$ for a power of 80%. These calculations revealed that the sample size of 33 for TEOAE test was adequate to show effects of approximately 1 dB with the chosen statistical power.

Thirty-five participants (20 female and 15 male) in the age range 18- 30 years (mean= 24.9 years) completed all the testing in this study. Three additional participants did not complete the testing. All testing (except ERP, see below) was carried out in a sound-treated room satisfying criteria in ISO 8253-1 for air conduction audiometry using earphones down to 0 dB HL. ERP was conducted in a quiet office room.

The experiment was approved by the ISVR Human Experimentation Safety & Ethics committee, University of Southampton. One internal and one external risk assessment was completed prior to applying to Safety & Ethics committee. The experiment was also insured via University of Southampton Research Office. All participants signed informed consent forms[2]. An example of this form is shown in Appendix 3.2.

3.2.2. Test protocol

This study was carried out in the following steps;

- Acceptance of participants according to sample size calculations and selection criteria, and completing the pre-experimental formalities.
- Baseline and post-exposure audiological measurements: the order of pre-exposure measurements was ERP, DPOAE (DP-gram and DP growth) and CAS-TEOAE, while the order of post-exposure measurements was reversed. OAE were recorded immediately before and after the exposure in an attempt to not to miss any subtle transient changes. The idea was to perform OAE tests

[2] Similar forms were used in other experiments.

immediately before and after the exposure in order to maximise the sensitivity to detect small changes, if any.

- Exposure to mobile phone EMF: during one test session the exposure system was effective (genuine), and during the other it was ineffective (sham exposure), blind to the participant and experimenter (double-blind). The order of genuine and sham exposures was random and counter-balanced.
- Audiometry was conducted at the beginning and at the end of each test session. The participants were advised to report immediately if they suspect or experience any ear and hearing related problems due to the exposure. No one reported any health effects due to the experimentation.
- Each participant was strongly advised (in written) to report to the tester and/or supervisor of the project and their respective General Physician (GP) immediately, if he/she experienced any hearing, balance or tinnitus problems following their participation in the experiment. The participants were also contacted by email after the experimentation to report any hearing health problems. There was no such incident reported by any participant.

Each test session took approximately 150 minutes. Figure 3.1 shows the block diagram of the study protocol. Exposure in one of the sessions was genuine while the other was sham.

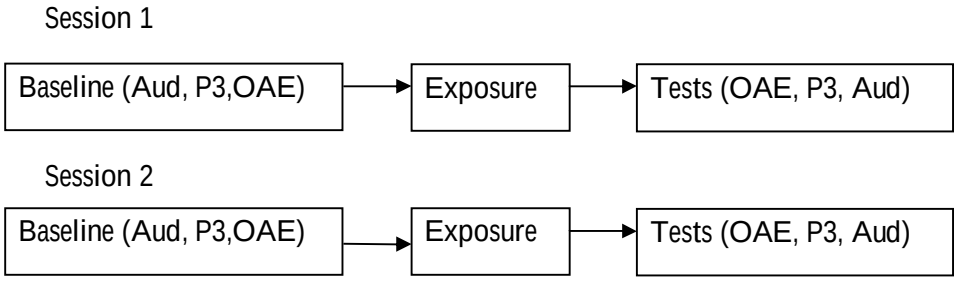

Figure 3.1. Block diagram of the study protocol.

3.2.3. Contralateral suppression of TEOAE (CAS effect)

TEOAE were recorded using the Otodynamics ILO 292. TEOAE were obtained in a linear mode with stimuli consisting of clicks of 80 μs duration. The stimulus was presented at five different levels (3 dB steps between the different intensities), with intra-meatal intensities from 57 to 69 dB pe SPL, with a click rate of 50/s and post-stimulus analysis in the range 2- 20 ms. The order of the different click intensities was randomized to avoid order effects. Responses to a total of 260 sets of clicks were averaged above the noise rejection level of 47 dB. A TEOAE was defined if its amplitude was 3 dB above the level of the noise floor, with overall reproducibility 80% or more, and no bands less than 75% in four successive frequency bands ranging from 1 to 4 kHz. The ILO292 averages into two alternative buffers, A and B. Reproducibility is defined as the zero-lag correlation coefficient between A and B buffers. Noise is estimated from the A-B difference waveform and signal is estimated from the (A+B)/2 waveform.

The contralateral stimulation consisted of 35 dB SL white noise, generated by the ILO292 system, by means of Otodynamics alternating protocol (called difference on/off): 6 epoch of 80 clicks (3 with and 3 without CAS) = 480 response averaged (240 with and 240 without). CAS effect (in dB) was calculated by subtracting the amplitude of TEOAE with noise from that of without contralateral noise.

3.2.4. DPOAE

DP-grams were recorded with fixed frequency ratio (F2/F1=1.22) and plotted as a function of F2. F2 was swept from 2-6 kHz in 125 Hz steps. At each step, the signal was averaged for 90 epochs or until a minimum SNR of 15 dB reached. DP-grams were recorded with two L1/L2 combinations: 60/50 and 50/ 40 dB.

DP input/output functions were measured at F2 = 2 and 4 kHz, with the same frequency ratio F2/F1 of 1.22. The combinations of L1 and L2 were 50/35, 55/40, 60/50, 65/60, 70/70 dB. These combinations approximate the “scissor-level” paradigm of Kummer *et al*. (2000), which distinguishes normal and abnormal cochlea optimally. For each step, measurement of the DPOAE utilised signal averaging for 90 epochs or until a SNR of at least 15 dB was reached.

A custom made DPOAE system was used for recording. The instrumentation for DPOAE has been previously described by Parazzini *et al*. (2005a, b) and Wilson and Lutman (2006). This instrument was readily available for the present experiment and a similar method for calibration was also adapted. Figure 3.2 shows a block diagram of the DPOAE instrument. Primary frequencies are denoted by F1 and F2, while primary levels are denoted by L1 and L2. An Etymotic microphone system containing microphone probe (Etymotic Research, ER-10B+) and a pre-amplifier (+40 dB) was used for recording ear canal sound pressure. Two Etymotic ER-2 insert earphones were used to deliver the primary tone stimuli to the participant via the probe tip snugly sealed in the participant's ear canal. The amplified signal from the microphone system was digitised (16-bit resolution, 32768 Hz sample rate) by external hardware containing A/D and D/A converter units (IHR, DSP remote converter module). This external hardware also generated the primaries. Signal processing using custom software running on a TMS-320 DSP card converted consecutive 62.5-ms epochs of the microphone signal to the frequency domain by performing FFT with a bin width of 16 Hz. The complex FFT was averaged after rejection of epochs in which the estimated noise level was greater than 10 dB SPL in the frequency range close to the DP. The amplitude and phase of the DP was estimated from the real and imaginary FFT components corresponding to the single bin centred on the DP frequency. Noise at the DP frequency was estimated by averaging the power in 10 spectral lines on either sides of the DP. Recording of a DPOAE for a particular frequency stopped after a minimum number of epochs had been acquired and a minimum signal-to-noise ratio (SNR) was reached; these minima were set at 20 epochs and a SNR of 10 dB. If neither criterion was met, averaging was curtailed after 50 non-rejected epochs. The equipment recorded the phase of the primaries in degrees and the amplitude of DP in dB.

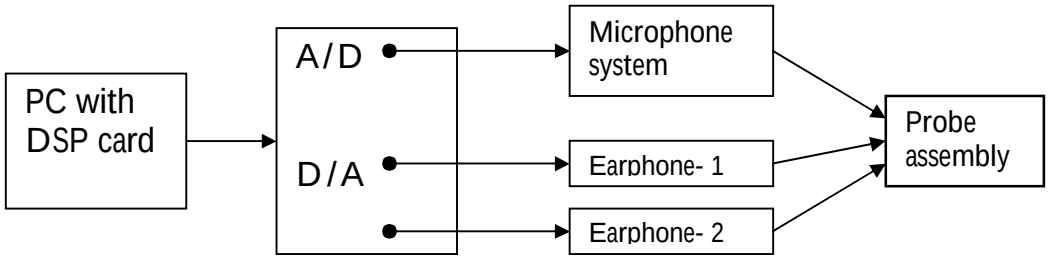

Figure 3.2. Block diagram of the DPOAE system.

The calibration corrections thus obtained were subsequently applied to stimulus levels produced by the earphones. The correction factors for intermediate frequencies were interpolated linearly. The probe microphone was calibrated at 1024 Hz only. Calibrating at 1024 Hz avoids the effects of standing waves at higher frequencies, which could potentially give a variable response depending on the position of the microphone within the ear simulator (or in the real ear). The frequency response of the microphone (Etymotic Research, ER-10B+) is approximately flat over the range of frequencies used and hence the single correction factor was applied to the entire frequency range.

3.2.5. Auditory event related potentials (ERP)

Cognitive event related potentials were recorded using a commercial AEP system (EP25, Interacoustics) using the stimulus oddball paradigm[3]. Disposable snap electrodes were placed on appropriate positions after cleaning the electrode site. The vertex (Cz) site was denoted as non-inverting or positive. Participants were asked to count the number of deviant stimuli. The electrode montage, stimulus and recording parameters are reported in Table 3.1.

Each recording was replicated twice. For the standard waveform the N1 and P2 peaks were considered while for the deviant waveform N2 and P3 peaks were marked. The N1

[3] Oddball paradigm means presenting two stimuli to pre-determined criteria such that one repeats frequently while the second tone occurs rarely. For example, in 100 presentations, if the standard to deviant criterion ratio is 4:1, one might occur 80 times and the other only 20 times. The order of occurrence of these stimuli is random. Usually, the subject's task is to attend to the odd stimuli either by counting or by pressing a button.

peak was identified as the most negative tip of the waveform occurring in the range 60-150 ms post-stimulus. For identifying the P2 component, the positive wave in the latency region between 120 and 220 ms was taken. N2, the negative peak and P3, the positive peak were identified in the deviant waveform in the latency regions of 150-240 ms and 230-450 ms respectively. These latency regions were considered based on observations from the literature (see McPherson, 1996, for review) and from looking at few pilot recordings.

Table 3.1. . Protocol for recording ERP.

Stimulus	
Type	Tone burst
Duration (rise/ fall; plateau)	10ms; 30ms
Frequency (standard; deviant)	1000 and 1500 Hz
Repetition rate	1.1/s
Intensity	70 dB nHL
No. of averages (standard)	250
Standard/ deviant ratio	4
Order presentation	Random
Presentation	Binaural
Transducer	Insert earphones
Acquisition	
Analysis time	−30 ms to 630 ms
Sample points	512
Amplification	50,000
Sensitivity	100 microvolts
Filters (band pass)	1-30 Hz
Electrode montage	
Channel 1	Cz- A1
Channel 2	Cz- A2
Ground	Fpz
Impedance	
Inter-electrode	Max 2 kΩ
Intra-electrode	Max 5 k Ω

Most of the times the peaks were clear but in few waveforms it was not possible to identify the peaks very clearly. Therefore, for positive peaks, the edge of the descending wave (not the peak) was located and the point of this edge was recorded from amplitude and latency marks. Similarly, for negative peaks the edge of the rising wave was considered for marking of peaks. If double or bifid peaks were observed in that latency range the most robust (positive or negative) point was recorded for amplitude, and the

mid-point between the two peaks was recorded for latency. The peaks were marked by a single tester usually; however, in occasions when a waveform was not clear a second observer was sought for guidance.

3.2.6. Exposure system

The exposure system was developed and kindly provided by a partner institution in the EMFnEAR project group. The exposure consisted of speech at a typical conversational level delivered via an ER-3A insert tube to one ear, and phone radiation exposure in either genuine (test) or sham (control) conditions. Genuine and sham exposures were on separate days (at least 24 hours apart) in a double-blind design.

The technical descriptions of the exposure system are adapted from the EMFnEAR project documents D21-D23. The phone radiation exposure utilised the normal output of a consumer mobile phone (Nokia 6650) at full power for 20 minutes. The participants received the phone radiation exposure at approximately 2 GHz (full power = 70 mW). The phone was connected by serial cable to a PC and controlled by special software provided by Nokia. The sham or genuine exposures were realised using a ''load'' or a ''dummy load''. For this purpose an external power load was connected to the remote antenna connector of the phone. A 50-Ω resistive load and an open-circuit dummy load were developed for sham or exposed conditions with the same shape and structure. In order to confirm the effectiveness of the load, surface scanning of the phone by near field measurement was performed by the partner institution in Hungary (National Research Institute for Radiobiology and Radiohygiene, Budapest). The ''load'' intercepts the RF signal to the internal antenna on the phone and dissipates the RF in the load, while the ''dummy load'' looked identical but does nothing to allow the RF to reach the antenna. No radiated RF fields could be measured using the RF load connected to the external antenna output, confirming its effectiveness for the sham exposure. The SAR distribution within a head phantom with the genuine exposure is shown in Figure 3.3. Two separate identical phones were used, one with the load permanently attached (sham) and the other with dummy load (genuine). Both phones have identical appearance and the allocation of the loads was unknown to the experimenter: they were simply labelled A and B (until completion of experimentation and statistical analysis). Figure 3.4 shows the Nokia 6650 mobile phone used in this study.

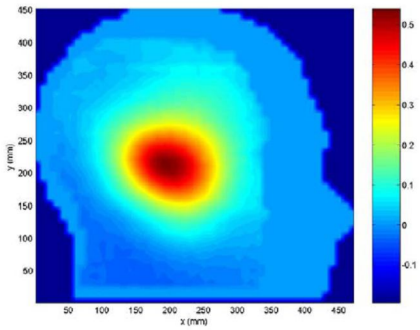

Figure 3.3. SAR distribution of the phone (adapted from EMFnEAR project document D21-D23). The central red region is the area of highest energy deposition.

Figure 3.4. Nokia 6650 phone with RF adaptor.

An arrangement for phone fixation that allows free head movement by the user had been designed and developed for a previous project examining the effects of radiation from GSM phones (Parazzinni *et al.*, 2005b) and was used for the present study (Figure 3.5). The positioning holder has three parts: a headband, an adjustable arm and a phone holder. All parts of the positioning system were made of non-metallic plastic materials in order to avoid any perturbation of the EMF emitted by the mobile phone. The headband allowed free movement of the head without any disturbance of the phone from the adjusted position. By using the adjustable arm the phone could be placed as required and adjusted according to the size of the participant under investigation. The adjustable arm can be placed on either side of the headband. The phone holder was attached to a bracket glued to the battery cover of the phone, which is on the reverse side from the keypad. During the exposure the phone was placed such that its longitudinal axis followed an imaginary line from the entrance to the ear canal to the corner of the mouth, in accordance with the CENELEC standard EN 50361.

Figure 3.5. Arrangement for positioning the mobile phone. (Reproduced with permission from the EMFnEAR project document D21-D23).

It is important to note that this is the same exposure that a user would receive by using the phone to make a normal call for 20 minutes at the limit of the range of the nearest base station in the cellular phone network. Therefore, the exposure was well within the limits of operation of the mobile phone in normal use and for which it has obtained CE approval. Normal users may experience this amount of exposure on a daily basis.

3.2.7. Statistical methods

The statistical analysis of the data was performed blindly. The Kolmogorov-Smirnoff test was performed on the CAS-TEOAE, DPOAE and ERP data collected on each session to check the distribution of the data. The K-S test showed that the raw data were normally distributed; hence, parametric statistics were applied. Representative histograms are presented in Appendix 3.3. The test-retest repeatability of the data was determined by intra-subject SD on replication. This was computed by dividing the standard deviation of the difference between the measures obtained in the two pre-exposure test sessions by $\sqrt{2}$. The reason for dividing by $\sqrt{2}$ is because the standard deviation of the difference includes the pooled uncertainty of the two measurements and if each replication has the same uncertainty (intra-subject variance) the difference has double the variance. The repeatability of the various measures is thus expressed in term of replication SD calculated in this way.

Repeated measures of analyses of variance (RM-ANOVA) were performed to examine if there were any significant changes due to mobile phone exposure. RM-ANOVA focused on within-subject variations rather than the differences between participants. Time of testing (pre- and post-exposure) and phone session (real and sham) were the two within-

subject factors, with no between-subject factor. Greenhouse-Geisser correction was applied for sphericity where required and Bonferroni adjustment was selected for multiple comparisons. The statistical package used was SPSS for Windows version 15.0, which does not allow performance of post-hoc tests as there are no between subject factor. As a result 'simple' contrast within reference category was used to analyze if there were any significant paired differences. Additionally, a paired sample *t*-test was used to further confirm the results. Whenever a paired sample *t*-test was used, it was performed to check the difference between 'change' in real and sham phone sessions. Change in a given measure in real or sham phone session was determined by subtracting the pre-exposure measure from the post-exposure measure for that particular session. This also allows control for the slight change (if any) between the baseline measurements of the two phone sessions. The basic level of significance was always set at 0.05.

RM-ANOVA was performed separately for CAS-TEOAE, DP-gram, DP growth and ERP. For DP-gram, RM-ANOVA was performed separately for the two primary levels (L1/L2= 60/50 and 50/40 dB) because DPOAE were present unequally for the two levels. Combining these two levels into single analysis would have reduced the sample size as RM-ANOVA removes cases with any missing values. For the very same reasons, analysis for DP growth was performed separately for 2 and 4 kHz and also separately for the three primary tone level combinations (L1/L2= 50/35, 55/40 and 60/50 dB). For DP growth at L1/L2= 65/60 and70/70 dB, RM-ANOVA was performed with two additional within-subject factors; frequency (2 and 4 kHz) and L1/L2 level (65/60 and 70/70 dB). For ERP, RM-ANOVA was performed separately for each peak (N1 and P2 for standard, and N2 and P3 for deviant waveform) and also separately for latency and amplitude. For example, RM-ANOVA for N1 latency was performed with two within-subject factors (time of testing; pre-vs. post, and phone session; real vs. sham) with no between-subject factor.

For DP growth and ERP, change in a given measure in real and sham phone exposure session was computed. If phone exposure has any effect, then the change in the genuine exposure session will be different from that in the sham exposure session. RM-ANOVA was performed on these values with within-subject factor phone session (real and sham). For DP growth with L1/L2= 65/60 and 70/70 dB, two additional within-subject factors frequency (2 and 4 kHz) for L1/L2 level (65/60 and 70/70 dB) were considered.

3.3. Results

3.3.1. Intra-subject repeatability

The mean and replication SD of the TEOAE amplitude, CAS effect of TEAOE, and DPOAE in dB SPL are presented in Table 3.2. The TEOAE data presented here are obtained by averaging the amplitudes from five different levels from the no-noise recordings. The mean and replication SD of ERP latency in milliseconds (ms) and amplitude in microvolt (μV) are given in Table 3.3. The N1 and N2 peaks have negative voltage. The mean in both Table 3.2 and 3.3 was calculated by averaging the means of the two pre-exposure sessions. From the Table 3.2, it appears that the amplitude of DP-gram recorded at low primary levels (L1/L2 = 50/40 dB) was not very stable across sessions. Similarly, the DP growth recorded at low primary levels (L1/L2= 50/35 and 55/40 for 2 kHz and 50/35 for 4 kHz) was not very repeatable.

Table 3.2. Mean (averaged pre-exposures real and sham) and replication SD (pre-exposure real vs. pre-exposure sham) of OAE.

Test		N	Mean	Replication SD
TEOAE		35	11.95	2.52
CAS		35	1.11	0.42
DP-gram				
60/50		35	7.53	1.80
50/40		34	1.35	2.41
DP growth	L1/L2 (dB)			
	50/35	27	0.01	1.98
	55/40	32	1.52	1.93
2000 Hz	60/50	34	2.21	1.91
	65/60	35	3.82	2.07
	70/70	35	6.74	2.49
	50/35	26	0.93	1.41
	55/40	28	3.48	1.25
4000 Hz	60/50	32	4.13	2.01
	65/60	35	4.82	2.87
	70/70	35	7.89	2.49

The latency and amplitude of ERP peaks were more or less stable across sessions as shown by the replication SD values. While the replication SD value for N2 peak shows stability across sessions, practically, marking of N2 peak was not always straightforward compared to other peaks. Paired samples *t*-test with Bonferroni adjustment for multiple comparisons was used to compare the difference between two pre-exposure sessions. Out of all measures, DP growth at 55/40 dB for 2 kHz was significantly different in the two pre-exposure test sessions ($p = 0.016$). This means that the mean DP growth at 55/40 dB for 2 kHz varies with test session.

Table 3.3. Mean (averaged pre-exposures real and sham) and replication SD (pre-exposure real vs. pre-exposure sham) of ERP.

	N1	P2	N2	P3
N	34	34	27	33
Latency (ms)				
Mean	87.16	162.13	217.4	305.71
Replication SD	7.01	18.9	23.24	25.9
Amplitude (μV)				
Mean	2.71	3.53	2.15	6.01
Replication SD	0.96	1.53	1.2	2.81

3.3.2. Audiometry

The air conduction hearing threshold level (HTL) shifts across subjects before and after real or sham exposure as a function of the audiometric frequencies (0.5 and 8 kHz). Figure 3.6 shows the mean HTL shift for the real and sham phone exposure as a function of frequency. The zero line represents no change. The general trend was a worsening of the HTL (i.e. an increase of the HTL) after a real exposure compared to the sham; the largest mean shift for real exposure was less than 3.5 dB HL. Two statistically significant differences contrasting sham and real exposure were found, specifically at 6 and 8 kHz ($p=0.05$); importantly, these differences remained significant even after the correction for multiple comparisons was applied.

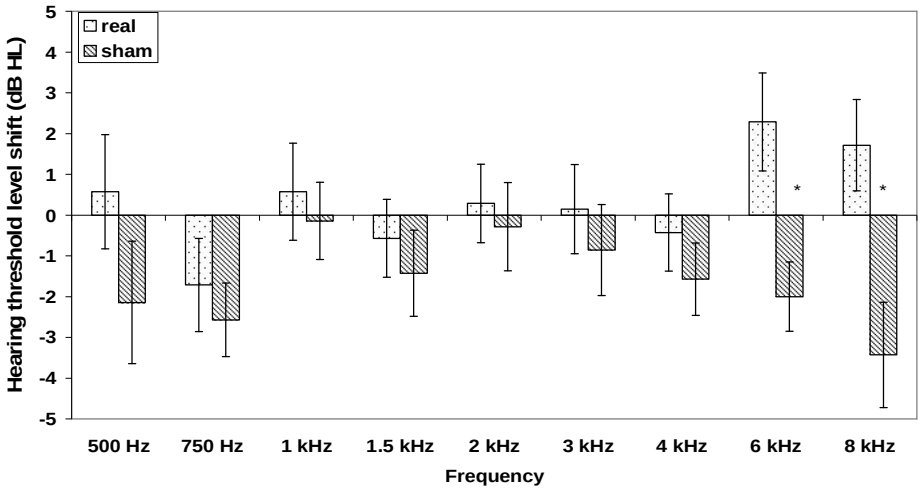

Figure 3.6. Mean HTL shift across subjects (n=35) for real and sham phone exposures. The error bars indicate ±2 SE and asterisk (*) indicates significantly different ($p<0.05$) between real and sham exposure.

3.3.3. TEOAE and CAS-TEOAE

TEOAE (without and with noise) were present in all participants in all test recordings. TEOAE (without contralateral noise) recorded at five different levels were averaged to get a single amplitude value. The mean amplitude and 95% confidence interval of TEOAE recorded in various sessions are shown in Figure 3.7. RM-ANOVA showed significant overall main effect ($p=0.031$) only for session (pre-post) but no effect for phone exposure or interaction of phone exposure and session. Further comparison by contrast method and paired sample *t*-test revealed that amplitudes in post-exposure (mean=11.23 dB; SD= 4.05) were reduced compared to pre-exposure (mean=11.6; SD=4.39) in the real phone exposure session; however, this effect was not significant ($p\geq0.05$).

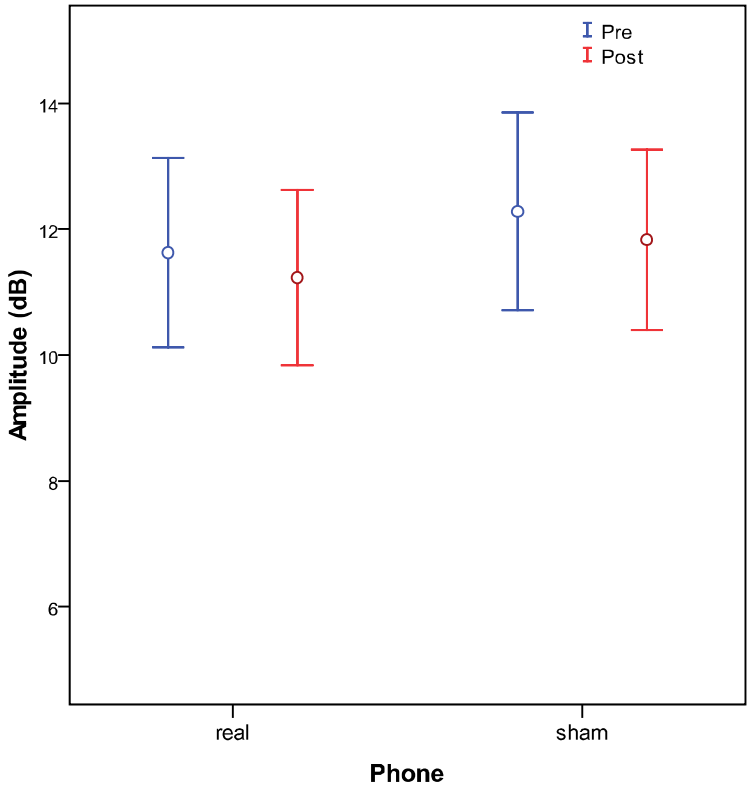

Figure 3.7. Mean and 95% confidence intervals for mean of amplitudes of TEAOE (without contralateral noise). Pre and post indicate pre- and post exposures respectively.

An example of a CAS-TEOAE recording is presented in Figure 3.8. The CAS effect recorded at the five levels (57 to 63 dB) was averaged to get a single amplitude value per recording in an attempt to make it a more stable measure. Figure 3.9 shows the mean and 95% confidence interval of the mean for the CAS effects. The change in CAS effect is the difference in CAS effect on TEOAE before and after exposure for a given session. For example, change in CAS effect in the real phone exposure session was calculated by subtracting the CAS effects of the pre-exposure from that obtained in post-exposure for the given session. The mean changes in phone real and sham exposure session were −0.005 dB (SD= 0.42) and −0.12 dB (SD=0.39) respectively. The negative sign means reduction in the amplitude of CAS-TEOAE.

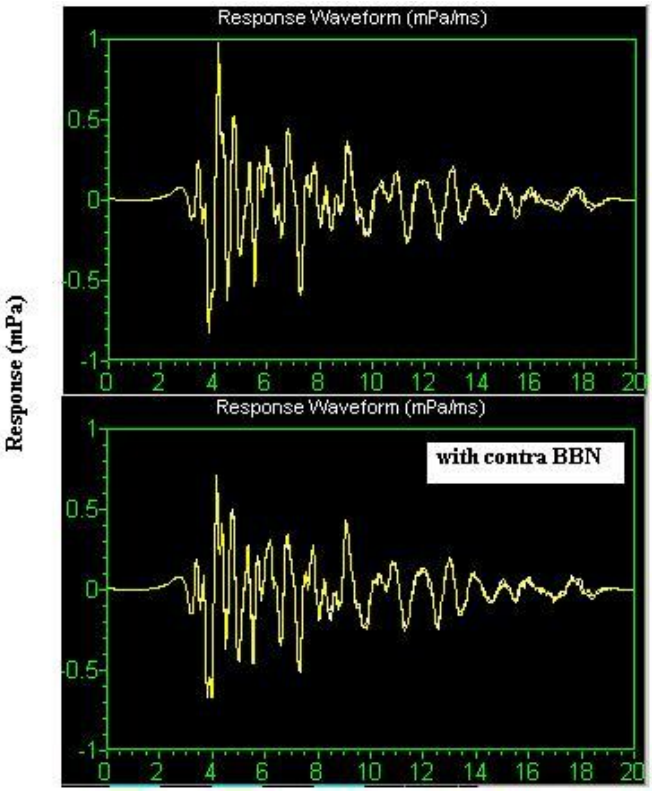

Figure 3.8. An example of a TEOAE waveform. Waveform shows without (upper panel) and with 60dB contralateral noise (lower panel) recorded in a real exposure session.

These values close to zero show that there was no change in either session. RM-ANOVA performed on CAS data revealed that phone session (considered factors are time, phone session and their interaction) has a very weakly significant overall main effect ($p = 0.044$), with more suppression in the real phone session than in sham exposure session. However, when further analysed by contrast method there was no significant effect of session $\times$ phone interaction and also a paired *t*-test showed no significant effect of phone in any sessions in Figure 3.9. In summary, paired *t*-test compared change in suppression in the real phone exposure session with that in the sham exposure session. The RM-ANOVA results is thus not meaningful considering the main aim of the study was to investigate the detrimental effect of phone exposure. Additionally, the TEOAE collected without contralateral noise when analysed did not show any significant effect of phone exposure on its amplitude ($p \geq 0.05$). Hence, it can be interpreted that there was no detrimental effect of phone exposure on amplitude of the CAS-TEOAE.

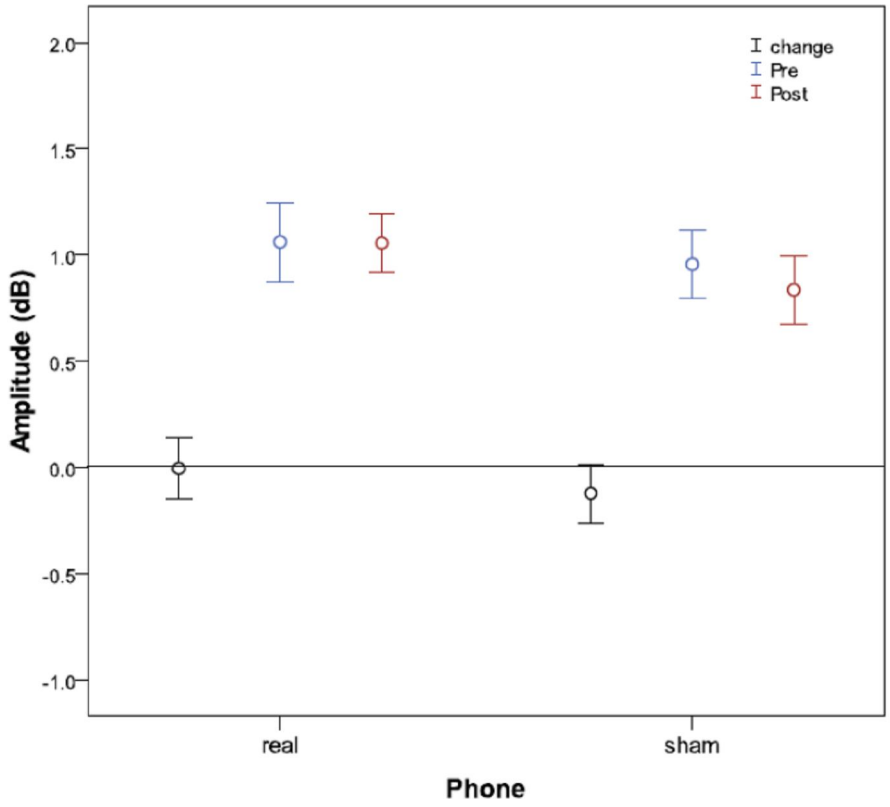

Figure 3.9. Mean and 95% confidence intervals for mean of CAS effect of TEAOE. Pre and post means pre- and post exposures respectively. Change is the difference between post- and pre-exposures for a given phone session.

3.3.4. DP-gram

Figures 3.10 and 3.11 show examples of a DP-gram recorded at L1/L2= 60/50 and 50/40 dB respectively, plotted as a function of F2 frequency. It appears that the emissions are reduced for L1/L2=50/40 dB compared to 60/50 dB, while the noise floor remained more or less the same at the two recording conditions for the same participant.

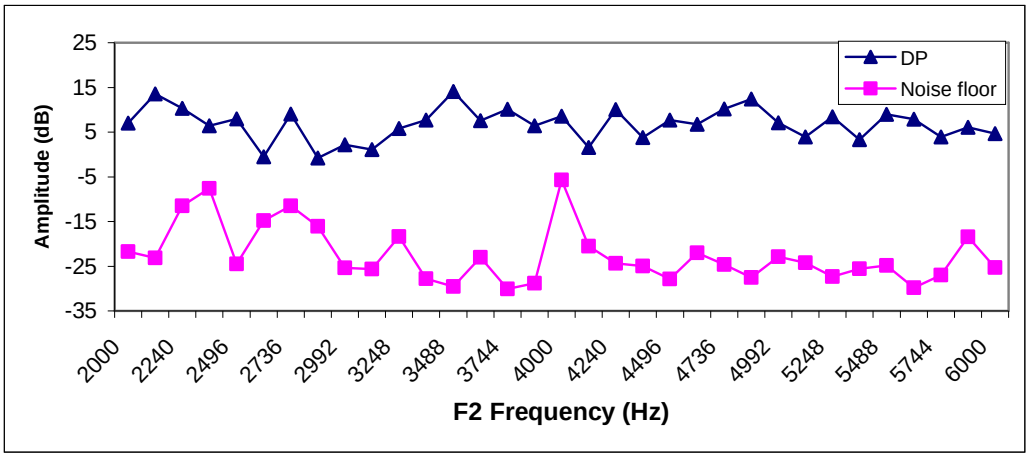

Figure 3.10. An example of DPOAE at L1/L2= 60/50 dB recorded from a participant before exposure in the real phone session. The top curve (filled triangles) is the level of 2F1–F2 DPOAE and the bottom curve (filled squares) is the corresponding noise floor.

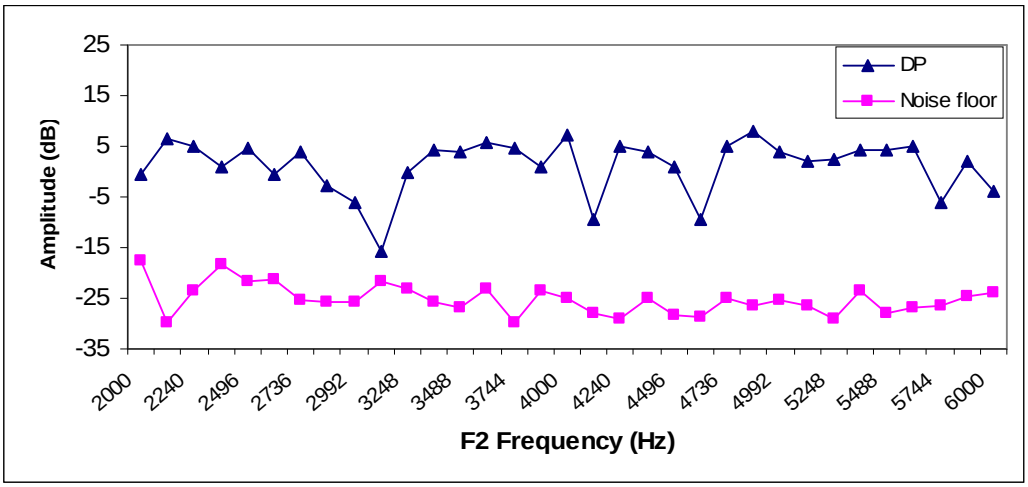

Figure 3.11. An example of DPOAE at L1/L2= 50/40 dB from the same participant before exposure in the real phone session. The top curve (filled triangles) is the level of 2F1–F2 DPOAE and the bottom curve (filled squares) is the corresponding noise floor.

DPOAE recorded at L1/L2= 60/50 dB were present in all participants, whereas, DPOAE recorded at L1/L2= 50/40 dB were present in 34 (out of 35) participants and were usually very low in amplitude. It is important that when DPOAE were present, they remained so in all the sessions. The instability of the amplitude of the DPOAE at L1/L2= 50/40 dB is also shown by the low mean value compared to replication SD (Table 3.2). The amplitudes of DPOAE across frequency were averaged to obtain a single amplitude value. Amplitude shift for a given phone session (real or sham) was

calculated by subtracting the DPOAE amplitude in pre-exposure from that in the post-exposure. Figure 3.12 shows the mean and 95% confidence intervals for change in amplitude of DPOAE in real and sham phone exposure sessions at L1/L2= 60/50 and 50/40 dB. RM-ANOVA performed separately for the two primary levels (L1/L2= 60/50 and 50/40 dB) showed that none of the within-subject factors (time and phone exposure session) had a significant effect, nor was there a significant interaction. Thus, exposure to mobile phone did not have any effect on the amplitude of DPOAE recorded at L1/L2= 60/50 and 50/40 dB.

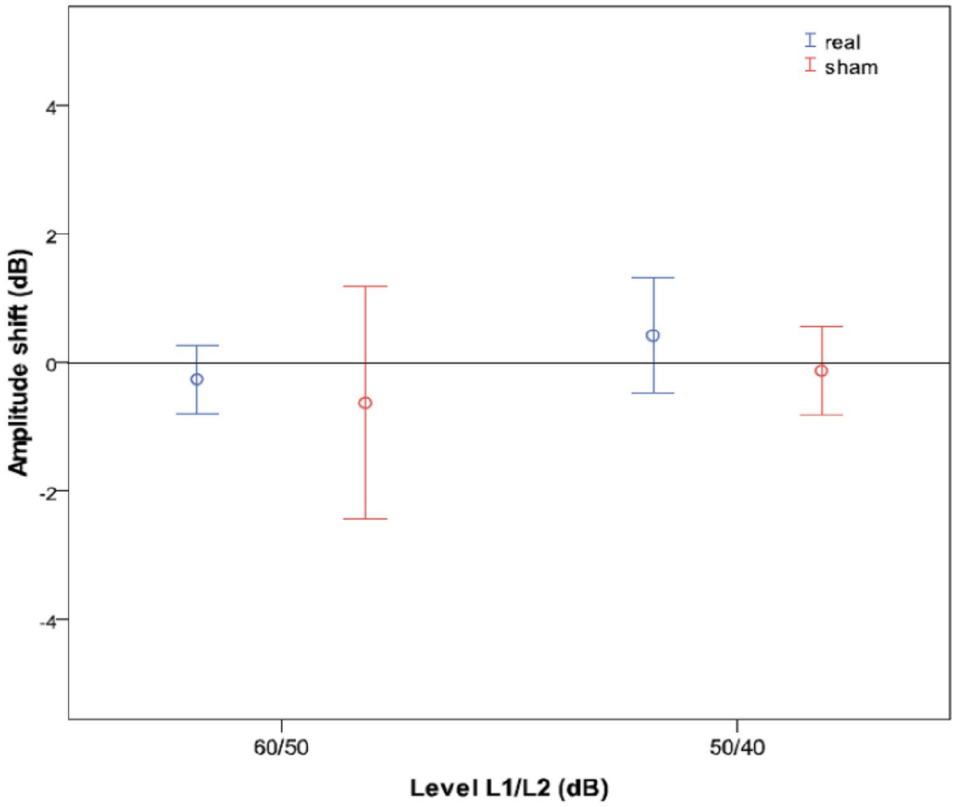

Figure 3.12. Mean and 95% confidence intervals of mean for change in amplitude of DPOAE in real and sham phone exposure sessions at L1/L2= 60/50 and 50/40 dB. Note that positive change indicates increase post-exposure relative to pre-exposure.

3.3.5. DP growth

The presence of DPOAE in a given participant was dependent upon primary level and frequency. The number of participants in which DPOAE were present at each level and frequency is provided in Table 3.2. An example of DP growth recording is presented in

Figure 3.13. In general, DPOAE were not present at low primary levels in all participants and the prevalence (presence or absence) of DPOAE did not change with sessions or pre- and post-exposures.

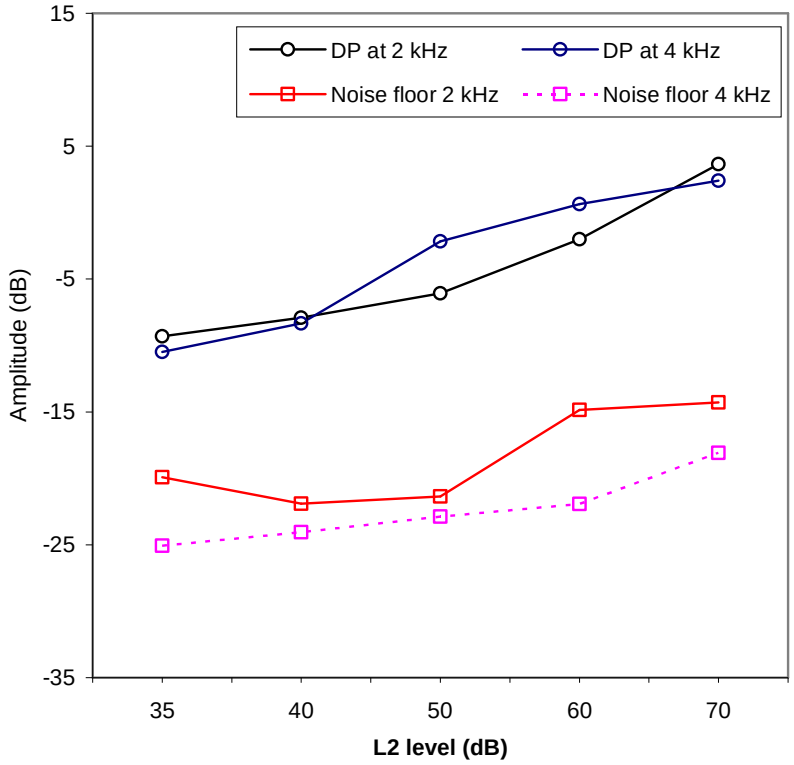

Figure 3.13. An example of DP growth recording plotted as a function of L2 level at 2 and 4 kHz from a participant before exposure in real phone exposure session.

Figures 3.14 (for 2 kHz) and 3.15 (for 4 kHz) present the mean and 95% confidence intervals of the amplitude of DPOAE and the change in amplitude of DPOAE (post-exposure minus pre-exposure) for real and sham phone exposure sessions. It appears from these figures that irrespective of the variations in amplitude of DPOAE across frequency the slope looks more or less similar at the various recordings of DPOAE.

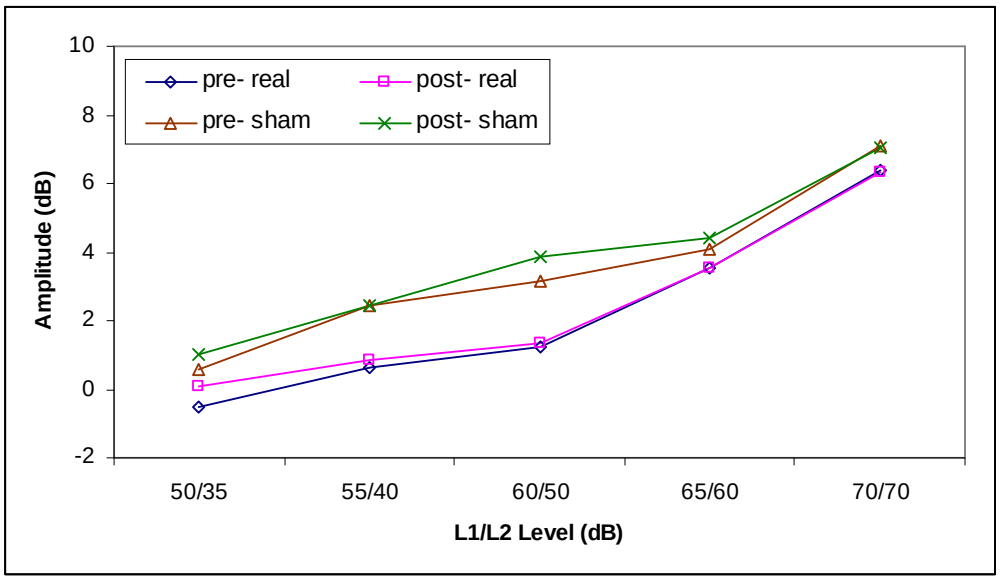

Figure 3.14. Mean amplitude of DPOAE at 2 kHz at various L1/L2 levels in real and sham phone exposure sessions. Pre-real, post-real, pre-sham, and post-sham respectively refer to pre-exposure real phone session, post-exposure real phone session, pre-exposure sham phone session, and post-exposure sham phone session.

RM-ANOVA showed a main effect of phone session on the amplitude of DPOAE for L1/L2 levels of 60/50 and 55/40 at 2 kHz only. The effect of phone was further probed by the contrast method and paired samples *t*-test (change in real and sham phone exposure sessions), which revealed that there was no significant effect of phone exposure in any sessions. On RM-ANOVA, the effect of phone is attributed to the difference between the two baseline measurements at 2 kHz. As expected, an effect of frequency and level was found. In general, the amplitude of DPOAE was higher at high L1/L2 levels and for 4 kHz compared to 2 kHz.

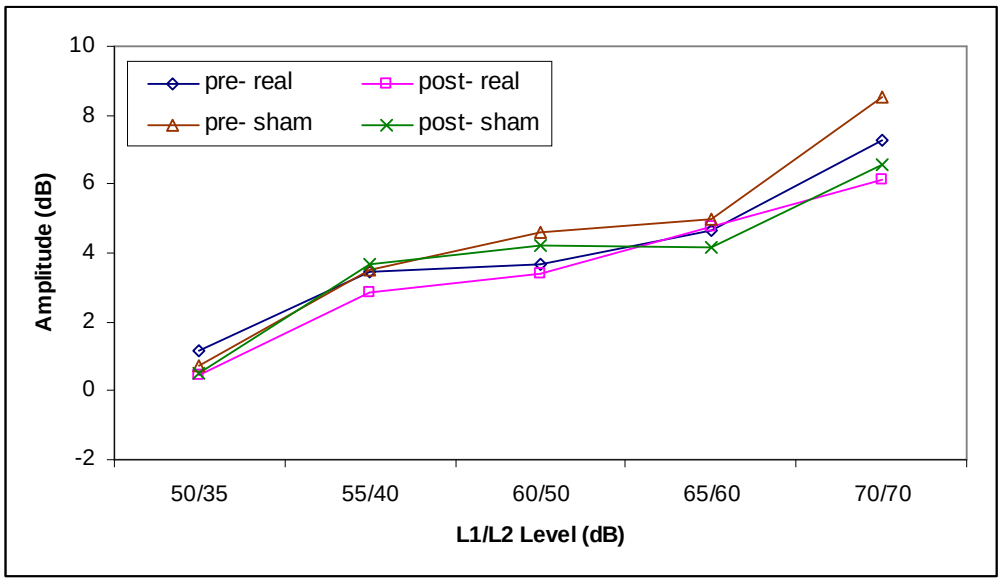

Figure 3.15. Mean amplitude of DPOAE at 4 kHz at various L1/L2 levels in real and sham phone exposure sessions. Pre-real, post-real, pre-sham, and post-sham respectively refer to pre-exposure real phone session, post-exposure real phone session, pre-exposure sham phone session, and post-exposure sham phone session.

To take care of the baseline shift between the two phone sessions, change in amplitude in real and sham phone exposure sessions was computed. RM-ANOVA was performed on these values with phone session (real and sham) as the within-subject. There is no significant effect of phone exposure on the DP growth measurements at 2 and 4 kHz.

3.3.6. ERP

The ERP standard and deviant waveforms were present in 34 and 33 (out of 35) participants respectively. In all participants, the N1 and P2 peaks in the standard waveform were distinct and clear, hence, were easy to mark. In contrast, even when the deviant waveform was present there was some uncertainty in marking N2 peak as it was not well defined and could be marked in only 27 out of 33 participants. In general, when a peak was present it remained so in all the four recordings. Figures 3.16 and 3.17 show the representative examples of the standard and deviant ERP waveforms respectively from one subject for different sessions. In general, it is evident from these figures that standard waveforms are clearer compared to the deviant waveform, which is expected because there were four times as many epochs in the standard waveform average. Figures 3.18 (for latency) and 3.19 (for amplitude) present the mean and 95% confidence

intervals of the ERP peaks. Figure 3.19 additionally presents the change in amplitude (post minus pre exposure) for real and sham exposure phone session.

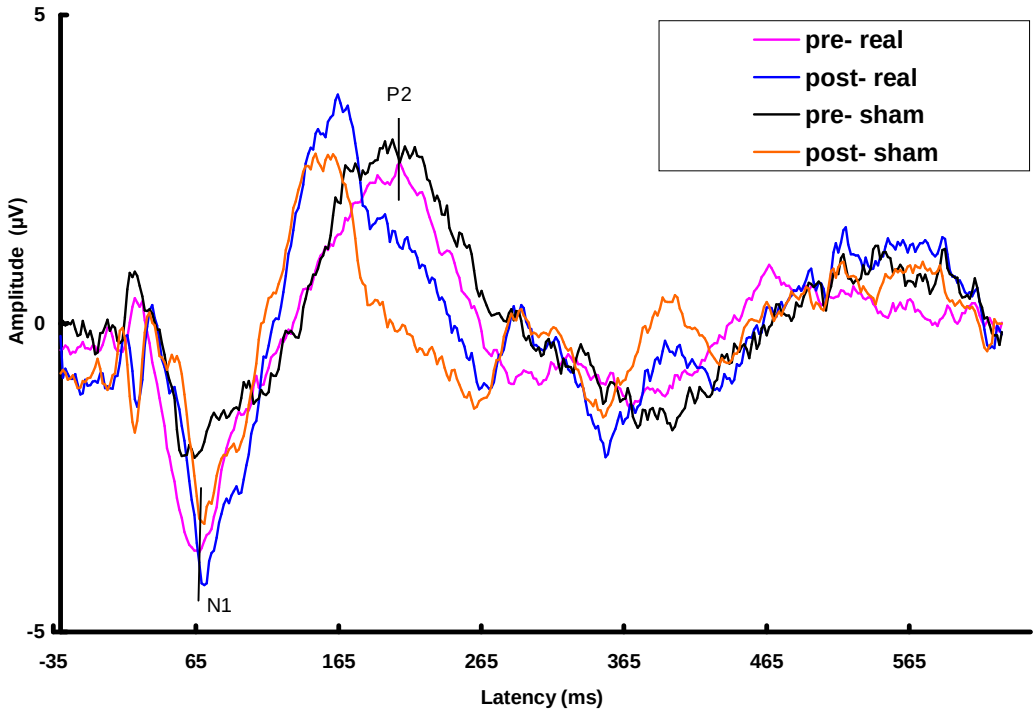

Figure 3.16. Example of standard ERP waveforms from one subject. Pre-real, post-real, pre-sham, and post-sham respectively refer to pre-exposure real phone session, post-exposure real phone session, pre-exposure sham phone session, and post-exposure sham phone session. N1 and P2 peaks are marked for pre-real exposure standard waveform.

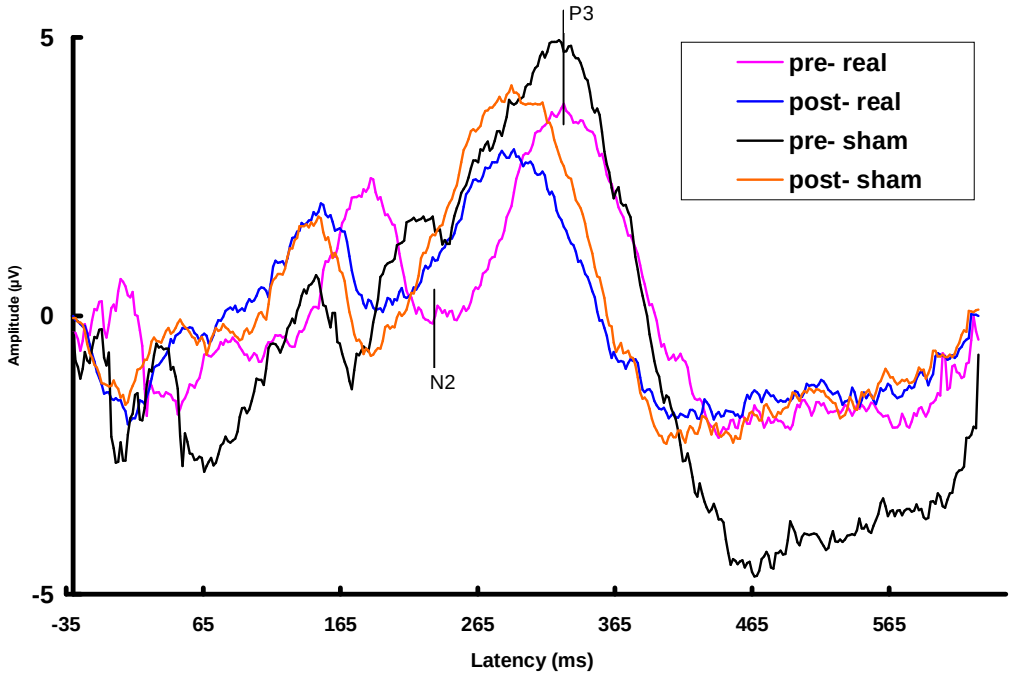

Figure 3.17. Example of deviant ERP waveforms from the same subject. Pre_real, post_real, pre_sham, and post_sham refer to pre- exposure real phone session, post-exposure real phone session, pre-exposure sham phone session, and post-exposure sham phone session respectively. N2 and P3 peaks are marked for pre-real exposure deviant waveform.

RM-ANOVA was performed separately for change in latency and amplitude for each peak, with a single within-subject factor phone session (real and sham) to examine the effect of phone exposure. There was no significant effect of phone exposure on the mean latency or amplitudes of all the peaks of standard and deviant waveform except for N2 amplitude (p=0.015). In the real phone session, the amplitude became more negative or increased (mean change= $0.51\ \mu V$, SD= 1.44) after exposure to the EMF from the UMTS phone, whereas, the N2 amplitude became less negative (mean change= $0.38\ \mu V$, SD= 1.48), i.e., amplitude decreased in the sham phone exposure compared to real phone exposure session. In view of the instability of the N2 peak (as it was not well defined in most of the times and, importantly, the N2 amplitude was significantly different in two pre-exposure sessions) the significant finding on N2 amplitude change may not be meaningful can be dismissed and in the present context.

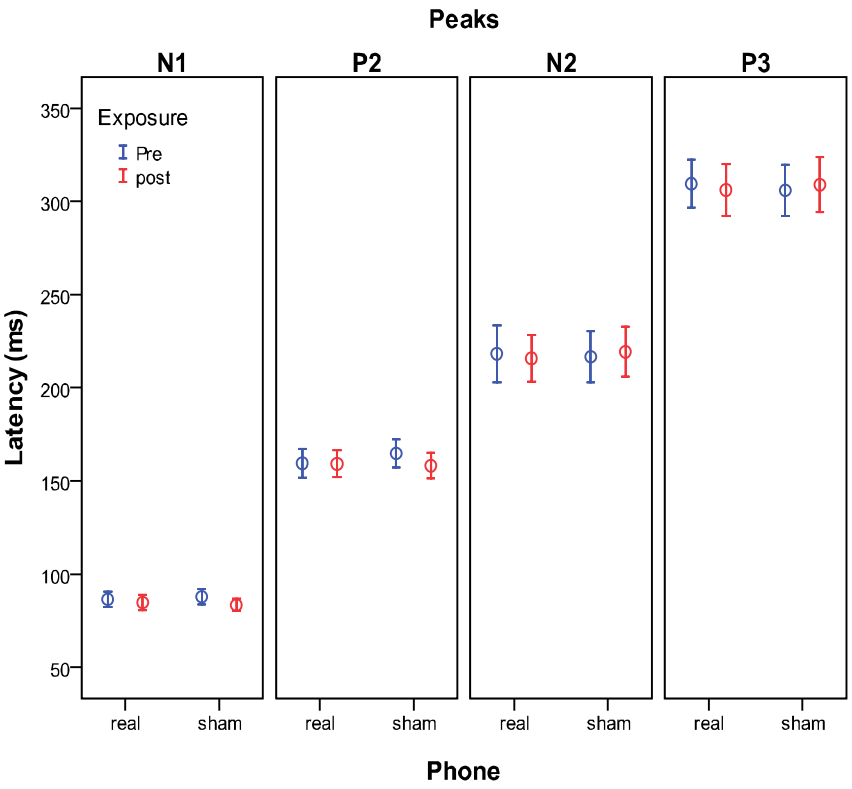

Figure 3.18. Mean and its 95% confidence intervals of latency of various ERP peak before (pre-) and after (post-) phone exposure sessions (real and sham). Note that N1 and P2 were from standard and N2 and P3 were from the deviant waveforms.

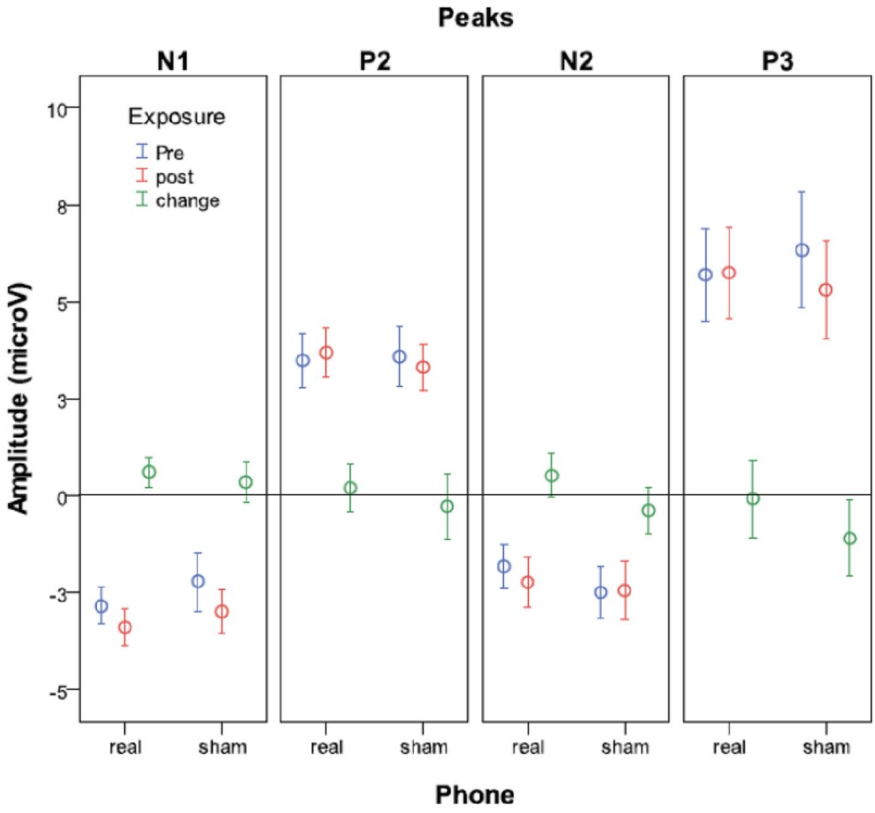

Figure 3.19. Mean and its 95% confidence intervals of amplitude of various ERP peak before (pre-) and after (post-) phone exposure sessions (real and sham). Note that N1 and P2 were from standard and N2 and P3 were from the deviant waveforms.

3.4. Discussion

Mobile phone use has become a necessity of modern life and communication. Davidson and Lutman (2007) have found extremely high prevalence of mobile phone usage among a student population in the UK. The main objective of this study was to investigate the potential immediate after-effects of UMTS phone exposure on human auditory functions. In addition to using sensitive tests, a comprehensive evaluation technique was used. For example, for the first time the possible effect of UMTS mobile phone exposure on the efferent auditory system was evaluated. To increase the sensitivity of the test methods DP growth functions were also examined. To date, while several studies (details in Chapter 2) have investigated the potential effects of GSM phone there is no report examining the effects of UMTS phone on human auditory function. This means that direct comparison of the present findings cannot be made with previous reports. The use

of a double-blind design is the main strength of the present study. In most studies evaluating short-term effects the typical duration of exposure is 10-15 minutes, in contrast, the present study used duration of 20 minutes in an attempt to increase the effect size and also simulate the real world usage time. The results are discussed in the context of findings from studies (with good design) on GSM phones. The present experiment also contributes to the EMFnEAR data (Parazzini *et. al.* Radiation Research, accepted). The results (except, audiometry) are consistent with the larger data pool of the EMFnEAR project.

3.4.1. Audiometry

Air conduction hearing thresholds provide a generic index of hearing status. The main goal of including audiometry in the test protocol was to recruit normal hearing participants in the study and also to examine any behavioural changes in hearing following UMTS phone exposure. There appears to be worsening of HTL at high frequencies (6 and 8 kHz) following exposure to UMTS phone. The mean reduction of 2.0-3.5 dB could be of serious concern if this is a real effect. It is quite hard to judge if the present effect is real although the reason for the source of any potential error or bias is not clear. Such an effect has not been reported before for GSM phones. Interestingly, the audiometry results do not corroborate any of other tests, particularly, TEOAE and DPOAE (discussed later in this section). Also, the effect was reduced to less than 1 dB when pooled with data (n=134) from the EMFnEAR project (Parazzini *et. al.* 2009). It is also important to note that the sample power calculation for this study was based on TEOAE results.

3.4.2. TEOAE and CAS effect on TEOAE

Analysis of amplitudes of TEOAE recorded in no-noise condition did not show any significant effect of phone exposure. The replication SD value of TEOAE found in present study is 2.52 dB and is consistent with a previous report by Hall and Lutman (1999) who found similar values (TEOAE=1.8 and MLS-TEOAE[4]=2.9 dB) in an attempt to detect changes in cochlear functioning from noise exposure. While the direct comparison of the finding on EMF exposure cannot be made with previous studies due

[4] TEOAE evoked by maximum length sequences paradigm.

to the nature of exposure and the TEOAE protocol, the lack of GSM phone effects on temporal and spectral fine structure of TEOAE found by Paglialonga *et al*. (2007) indirectly supports the present findings on TEOAE.

The possibility of effects of EMF radiated by UMTS phone on the efferent system was examined by using contralateral acoustic suppression of TEOAE. Good TEOAE recordings were obtained in all participants, which help with CAS measurement. CAS of TEOAE is thought to reflect the functioning of the efferent auditory system, in particular, the medial olivocochlear system (Berlin *et al*., 1993; Giraud *et al*., 1996; Ryan *et al*., 1991). The motivation to study the effects of mobile phone radiation on the efferent auditory system stems from a study that found decrease in otoacoustic emission suppression due to occupational noise exposure (Sliwinska-Kowalska and Kotylo, 2002) and also from large body of literature on auditory neuropathy[5] (Starr *et al*., 1996), where OAE are normal while the suppression of OAE is abnormal. This means that cochlear functioning as measured by OAE might be normal while the efferent system as shown by suppression measurements may not be normal.

The amplitude of TEOAE suppression in the two pre-exposure sessions is repeatable. The mean suppression of 1.11 dB is more or less consistent with previous studies on TEOAE suppression (Berlin *et al*., 1993; Giraud *et al*., 1996; Ryan *et al*., 1991). This agreement validates the accuracy of measurements. Present results suggest that there is no significant effect of mobile phone exposure on the amplitude of suppression of TEOAE. The changes in amplitude of CAS effect of TEOAE in real and sham phone exposure session are similar.

3.4.3. DPOAE

DP-gram and DP growth functions were recorded to study potential effects of UMTS phone exposure on cochlear functioning. DP-grams were recorded at low and moderate primary levels for a broader frequency range (2-6 kHz), while DP growth functions were established at several combinations of primary levels at 2 and 4 kHz. These combinations approximate the "scissor-level" paradigm of Kummer *et al*. (2000) and sensitively

[5] Auditory neuropathy is an auditory disorder characterized by normal OHC functioning (measured by OAE) and abnormal neural synchrony (as in ABR). In that population, usually OAE are present but there is no suppression of OAE with contralateral acoustic stimulation.

distinguish normal and abnormal cochlear function. The DP-grams recorded at low primary levels (L1/L2= 50/40 dB) were not very reliable as the mean amplitude was less than the replication SD (see Table 3.2), possibly due to poor signal to noise ratio. Analysis did not show any effect of phone exposure on amplitude of DP-grams recorded at either of the primary levels.

The DP growth functions are thought to reflect the compressive non-linearity of the cochlea (see Hall, 2000 for review, Kummer *et al*., 2000). Qualitatively, the shapes of the DP growth functions obtained in two pre-exposure sessions are consistent with previous studies (Moulin *et al*, 1992; Popelka *et al*, 1993). The notch (seen in Figure 3.14 and 3.15) seen at L1/L2= 65/50 dB for both 2 and 4 kHz found in the present study has also been previously reported. The notch at 2 kHz was not seen in one test session A (to recap, a notch was present in 2 out of 4 recordings); in contrast, the notch at 4 kHz appears to be stable and did not disappear with test session. The presence of these notches in DP growth function has been attributed to frequency shifts in DP fine structure (Popelka *et al*, 1993) and also to the presence of spontaneous emissions (Moulin *et al*, 1992). Amplitudes of DP at L1/L2= 50/35 and 55/40 dB for 2 kHz and 70/70 dB for 4 kHz were significantly different in the two pre-exposure test sessions indicating instability of DP amplitude at these levels. While the exact reason for this instability is unknown, it could be due to a mixture of variables related to variations in day of testing and general instability of the measures. To control these variations across the two test sessions, the change in amplitude in each session was calculated and considered for analysis. The results show no effect of mobile phone exposure on amplitude of DP growth functions. In a well designed study, Parazzini *et al*. (2005b) reported similar results using GSM phone exposure duration of 10 minutes. They also used novel measures such as amplitude of wave- and place-fixed components and phase gradient of DPOAE in addition to the overall amplitude of the DPOAE.

3.4.4. ERP

The ERP traditionally reflects perceptual and cognitive processes resulting from higher brain function in response to an auditory event. The mean values of amplitude and latency of various ERP peaks found in the pre-exposure sessions are consistent with the normative data summarised by McPherson (1996). Out of latency and amplitude of all

the peaks (N1, P2, N2 and P3), amplitude of N2 was relatively unstable across sessions. Although P2 (from Figure 3.16) appears to have a pre- vs post-exposure effect, analysis showed no effect of EMF exposure radiated by UMTS phone on latency and amplitudes of various ERP peaks except N2 amplitude. There appears to be no effect of exposure to EMF on the mean latency and amplitudes of the ERP peaks, suggesting no immediate after-effect on auditory cognitive functioning. The sporadic effect on N2 amplitude (increased negativity) in the real phone exposure session may be considered as physiologically or clinically meaningless in view of its high variability, and also because there was no effect on N2 latency. Moreover, the main goal of this study was to investigate the potential adverse effects of phone exposure not the potential beneficial effects of UMTS phone exposure. In contrast to the present finding, Hamblin *et al*. (2004) found effects of GSM phones on ERP. In their genuine relative to sham exposure, N1 amplitude and latency of standard waveform were reduced, with the reduction larger over midline and right hemisphere sites. P300 latency in the deviant waveform was delayed in the genuine exposure condition; however, as this difference was greatest at left frontal and left central sites (not at temporal sites) the interpretation of this result is unclear. Reaction time increased in the genuine relative to sham condition. No difference in task accuracy was found. The lack of effects on mean latency or amplitude in the present study could be due to differences in exposure type and duration, or electrode montage for ERP recordings (to recap, Cz was the only non-inverting electrode site in this study). An extensive electrode placement could not be used due to the limits of the instrumentation available and also in an attempt to reduce the total duration of each test session (which was already 150 minutes). If the effect on N2 amplitude is ignored there were no effects of EMF radiated by UMTS phone on the auditory cortical functioning as measured by ERP. Excluding the variability across test sessions, one might possibly argue in the lines of Hamblin and colleagues (2004) interpretation that EMF exposure could possibly lead to increase in accuracy of processing of auditory stimuli at the level of the supra-temporal auditory cortex and non-specific poly-sensory system. However, there is no evidence in the literature to support the mechanisms of such an interpretation.

3.5. Summary and Conclusions

With the increasing and inevitable use of mobile phones, there is also a growing scientific and public concern regarding the adverse hearing health effects of mobile phone use. The rationale for this research was based on the following observations and assumptions,

(i) Microwave hearing phenomenon, suggesting that pulsed electromagnetic fields could induce an auditory sensation, both in humans (Frey, 1962; Frey and Messenger, 1973) and in animals (Frey, 1967; Lebovitz and Seaman, 1977)

(ii) Mobile phone use necessitates holding the phone in close proximity to the ear, thus exposing the auditory system to the near-field of phone radiation.

(iii) Vulnerability of the auditory system to a variety of external and internal agents from noise to viruses.

The acute after effects of exposure to EMF radiated by UMTS phone was examined by a within-subject study in a double-blind design. The test battery aimed at sensitive and comprehensive evaluation of the auditory system. The tests included were audiometry, CAS of TEOAE, DP-gram, DP growth, and ERP. These tests were conducted before and immediately after 20-minutes exposure to EMF in 35 healthy young adults. The procedure was conducted twice in a double-blind design: once with a genuine (test) exposure and once with a sham (control) exposure. The administration of genuine and sham exposures was on separate days (at least 24 hours apart) and was counterbalanced in order, with the test participant and tester both blind to the condition being used. Results suggest that most of the measurements are repeatable except amplitude of DPOAE at low primary levels and the N2 amplitude of ERP. There was no potential adverse effect of phone exposure on any of the measures: amplitude of CAS of TEOAE, DP-gram, DP growth and latency and amplitudes of ERP peaks. Only, pure-tone audiometry showed two measurements that were statistically different at high frequencies (6 and 8 kHz) when sham and real phone exposures were compared. However, no other measures showed any sign of effect. It is important to note that no other measurements focused at high frequencies comparable to that of audiometry. A replication study with shift in PTA at high frequencies may help resolve this issue. Hence, exposure to EMF radiated by UMTS phones does not appear to have any effect on functioning of the efferent auditory system, cochlear functioning and non-linearity or auditory cortical functioning within the scope of the tests used in the present study.

CHAPTER 4

POSTURE INDUCED CHANGES IN COCHLEAR FUNCTIOING

4.1. Introduction

Manipulation of body position is one of the few non-invasive ways to induce changes in cochlear functioning. Alterations in posture induce changes in intra-cochlear pressure via changes in intra-cranial pressure. The intra-cochlear pressure alters cochlear function: (i) by acting directly on the cochlear structures (e.g., Chapman *et al*. 1990) and/or (ii) changing the stiffness of middle ear ossicles and inner ear (Böhmer, 1993). In principle, OAE can be affected by the changes related to forward transmission of the stimulus and also by reverse transmission from cochlea to ear canal.

DPOAE are generated from two different cochlear mechanisms (i.e., non-linear distortion and reflection). A review of literature (in Chapter 2) indicates that change in body position alters the amplitude of composite DPOAE. Alteration in the ICP (by changing body position) might have an effect on the generation mechanism of DPOAE. The present study was thus designed to examine the changes in the amplitude of DPOAE, its components, and the phase gradient of DPOAE in relation to changes in body position.

The separation of components into wave- and place-fixed components provides insight into generation mechanisms of DPOAE, while phase gradient measurements indicate the dominance of the components in DPOAE. Knight and Kemp (1999) show phase gradients centred on 2 kHz for various primary frequency ratios for a single subject. The steepest gradient, presumably corresponding to a place-fixed DPOAE, has a slope of approximately 2^{o}/Hz. This finding is corroborated by studies involving a larger sample size in our own laboratory (Wilson and Lutman, 2005). Arguably, the phase gradient associated with a place-fixed component at high frequency would be less steep due to shorter group delay at higher frequencies. The changes in phase gradient may be numerically small but could potentially represent a substantial change in the generation of DPOAE.

Change in body position alters the middle ear pressure and can induce changes in the middle ear sound transmission properties. Tympanometry was conducted to monitor the status of the middle ear. Tympanometry refers to the measurement of the admittance of the tympanic membrane and ossicles of the middle ear as a function pressure within the ear canal. The basic approach is to introduce a fixed probe tone (usually, 226 Hz at 85 dB SPL) into the ear canal where it will be affected by the admittance properties of the middle ear. This will be revealed as a change in the level of the probe tone as it is monitored by the probe microphone. Ear canal volume, static acoustic admittance and tympanometric peak pressure are the three commonly used parameters of the tympanogram. Ear canal volume is estimated from the admittance at a pressure of +200 daPa. Static acoustic admittance is the admittance of the middle ear at the peak of the tymapnogram. The pressure at which the static admittance is maximum (tymapnogram peak) is referred to as the tymapnometric peak pressure. Tymapnometric peak pressure is frequenctly used as an estimate of middle ear pressure. Clinical devices are usually calibrated in terms of admittance of an equivalent volume of air (cm^3). An example of a tympanogram from a participant is shown in Figure 4.1.

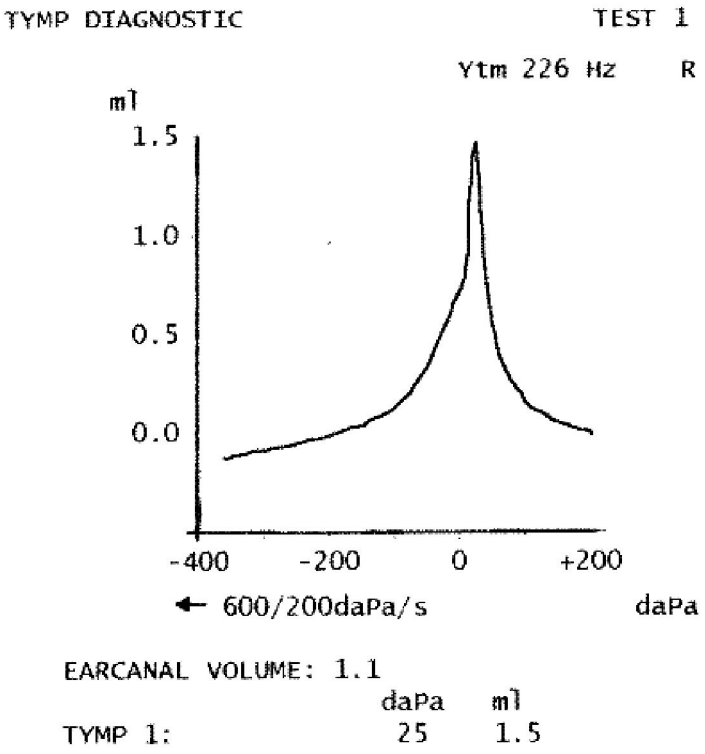

Figure 4.1. Example of a tympanogram (pressure= 25 daPa, static admittance =1.5 cm3).

4.2. Method

4.2.1. Participants

Twenty-one adults were screened for participation in this study. Of those, 15 were included and 6 were eliminated primarily due to low DPOAE level, history of noise exposure, ear wax, elevated audiometric thresholds and/or excessively high noise floor. Individuals were recruited via email and screened for hearing thresholds $\leq$15 dB HL between 0.25 and 8 kHz. Normal middle ear function was assessed by performing a tympanogram, which had to be type 'A', defined by static compliance between 0.4 and 1.5 cm^3 and peak pressure between ±150 daPa. There were 8 females and 7 males with a mean age of 24.6 years (range = 19-30 years); 7 left and 8 right ears. All tests were conducted in an acoustically treated double room setup. Audiometry and tympanometry were conducted prior to DPOAE measurements. The experiment was approved by the ISVR Human Experimentation Safety and Ethics committee, University of Southampton.

In order to ensure a high power (80%) for the experiment the required sample size was calculated using a sample power calculator. The standard deviations of multiple DPOAE measurements made within minutes of each other are generally less than 2 dB and often less than 1 dB (Voss *et al*., 2006). For this experiment an SD value of 1.5 dB was chosen to detect effects of at least 1 dB. The computation suggested 14 participants for the experiment with 80% power. However, it was decided to use 20 participants to maintain a high internal validity and power of the experiment.

4.2.2. Tympanometry

Tympanometry was performed using a calibrated commercial immittance meter (GSI 33, version I, Grason Stadler Inc.). Tympanograms were recorded in automatic mode using a 226 Hz probe tone at 85 dB SPL, with pressure swept from +200 to –300 daPa at a pump speed of 50 daPa/s. An appropriate probe tip was used depending upon the subject's ear canal size. Two recordings were made for each body position to determine the tympanometric peak pressure (daPa) and static acoustic admittance (cm^3). In most cases, the two recordings were very similar with little or no variation in tymapnometric

peak pressure (±5 daPa) and admittance (±0.3 cm^3). When there was a variation, the average of the two recordings was computed.

4.2.3. DPOAE measurements and data processing

DPOAE were measured using custom laboratory apparatus described in the previous (Chapter 3). The only difference to the instrumentation in the present experiment is the use of ER-10C system to deliver stimuli and record the OAE signals from the ear. DP-grams were recorded in response to two primaries denoted by F1 and F2 (F2>F1), and plotted as a function of F2 for a range of frequencies from 1000- 2500 Hz. The DP sweeps were collected with fixed frequency ratio (F2/F1= 1.22) while F2 was increased in 16 Hz step size. The levels of the two primary tones were: L1=60 dB, L2=50 dB.

The data processing was carried out mainly using Matlab software. The processing involved preliminary cleaning of the raw sweeps to get non-erroneous data for further processing with the three main goals: (i) phase unwrapping to adjust for abrupt changes in phase between successive data points (ii) unmixing for separation of components and (iii) averaging to get single amplitude value. A previous program was adapted as appropriate and used for the data collected in this experiment. The original and modified version of the program has been reported in Parazzini *et al*. (2005b) and Wilson and Lutman (2006). The Matlab scripts are reported in Appendix 4.1. The DP data were cleaned with a SNR criterion of 6 dB. The data points where the SNR was less than 6 dB, was defined as DPOAE absent and were not considered for further analysis. Most of the data points had SNR above the criterion; 5-6 data points were deleted in three cases. As primary frequencies were constrained to occur at 16 Hz intervals because of instrumentation restrictions, the frequency ratio varied slightly around 1.22 across sweeps. The rounding involved meant that certain steps in the sweep duplicated the same frequencies. These duplicates were also removed. The possibility of errors due to non-homogenous frequency spacing due to few data point deletion was avoided by linear interpolation of data to maintain the 16 Hz resolution.

Program 1: Data formatting and phase unwrapping.

The DP phase data obtained from the recordings were referenced to the phase expected for an instantaneous cubic nonlinearity, which is $2\phi_1-\phi_2$ for the 2F1–F2 DP, where ϕ_1 and ϕ_2 are the phases of the primaries F1 and F2 respectively measured by the probe microphone. Since phase can only occur within the range $\pm 180^o$, the phase data sequences were ''unwrapped'' to eliminate abrupt phase changes of more than 180^o between two adjacent data points. This was performed by either adding or subtracting multiples of 360^o in order to minimize successive phase steps. The frequency step size of 16 Hz between adjacent data points is marginally sufficient to prevent errors in phase unwrapping. The selected portions of the sweeps were saved in a suitable format for further processing. The unwrapped DPOAE phase data were plotted as a function of 2F1–F2 frequency. Phase gradient was defined as the slope of the best fit straight line. This gradient was expressed in degrees/Hz.

Program 2: Unmixing.

The unmixing program used a time-window separation according to the method described by Withnell *et al.* (2003). This method provided a time-domain representation of the DPOAE as shown in Figure 4.1. It then separated this into wave-fixed and place-fixed components of DPOAE based on latency. Short latency elements were considered as wave-fixed components while long latency parts were defined as place-fixed components. The time domain records generally show a peak before 2 ms and a series of peaks in the range 3–20 ms. The parameters used in this study were: cut-off time = 2 ms and recursive exponential filter order = 10. Previous studies along similar lines (Kalluri and Shera 2001; Parazzini *et al*., 2005b; Wilson and Lutman, 2006) that have used this technique have used a cut-off time of 2 ms based on data from Knight and Kemp (2001) and their own data. A cut-off time of ±0.5 ms (relative to 2 ms) was also explored here in a few recordings but did not produce any material change in the results. The cut-off time of 2 ms was suitable based on an observation of the time domain representation, which appears to drop to a minimum at around 3-4 ms. Figure 4.2 displays the schematic diagram of the important steps for separating the components of the DPOAE.

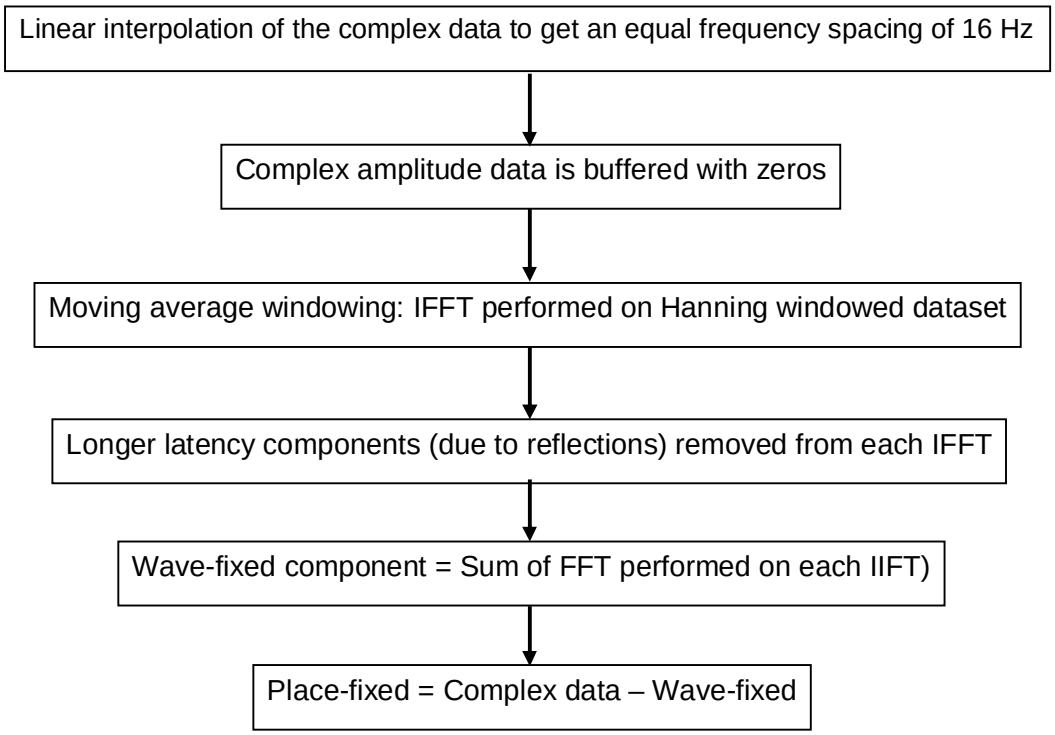

Figure 4.2. Schematic diagram of the steps for the unmixing program. This is a highly schematized diagram; please refer to the text for a complete description.

Briefly, the program converts the DPOAE magnitude and phase into their analogous time domain representation by performing an inverse Fast Fourier Transform (IFFT). To perform an IFFT, the complex data were linearly interpolated to get an equal frequency spacing of 16 Hz between any two adjacent data points. The complex amplitude data was then buffered with zeros to get an integer power of 2 data points. By not mirroring the complex amplitude data, this zero-extended data set represents the Fourier transform of an analytical signal. An inverse Fast Fourier Transform (IFFT) is then performed on 30 data-points wide Hanning windowed segments. The window moves each time by 15 data-points, so that it overlaps with the previous window. The program constructs a time domain representation of the total IFFT (sum of all individual IFFTs) as in Figure 4.3. Occasionally, in few recordings a peak was seen near the end of 62.5 ms in the time domain representation due to wrapping of the Fourier transform from the start of the waveform. In those cases, such peaks were therefore treated as wave-fixed components. Errors in the estimation of the two components have been previously described and

depend on how completely the components separate in time and how well the time-windowing can be applied to resolve the time differences (Kalluri and Shera, 2001). Separation of DPOAE into short and long latency components requires accuracy in choosing the smoothing function (or, equivalently, the shape and duration of the latency window). Ideally, the window should have a sharp cut-off in the time domain to cleanly separate components of different latencies and should avoid extensive spreading (or ringing) in the frequency response (smoothing function) (Kalluri and Shera, 2001). In an attempt to get these desired characteristics, each IFFT is subjected to a 'recursive exponential filter' (developed by Shera and Zweig, 1993) to remove longer latency components attributed to reflections within the cochlea. The recursive-exponential filters are entire functions and have no poles, discontinuities, or other undesirable features in the complex plane to contribute large oscillations to the smoothing function. An FFT is performed on each of these filtered IFFTs and then they are added to obtain the total FFT which gives the estimated wave-fixed component. The place-fixed component is estimated by subtracting the wave-fixed components from the original complex frequency domain data. The program output includes plots of the phases and amplitudes of both the estimated wave-fixed and place-fixed components in the frequency domain as a function of the DP frequency 2F1–F2, as shown in Figure 4.4.

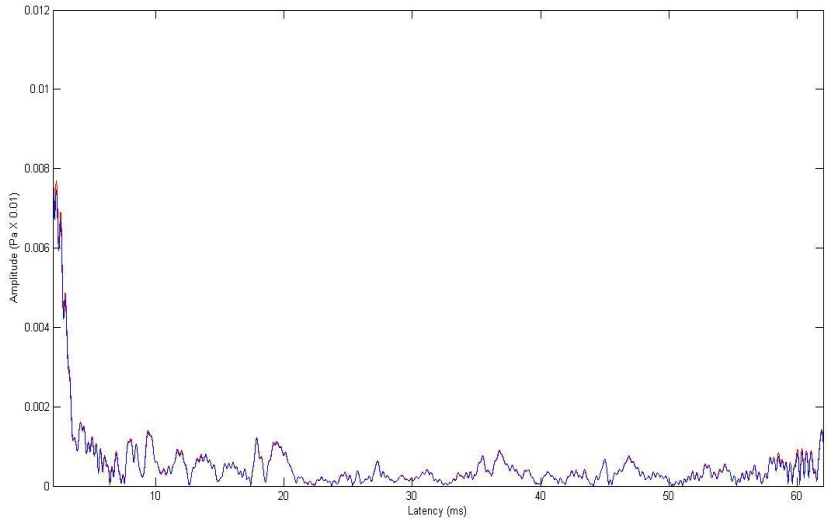

Figure 4.3. An example of time- domain representation of DPOAE after IFFT.

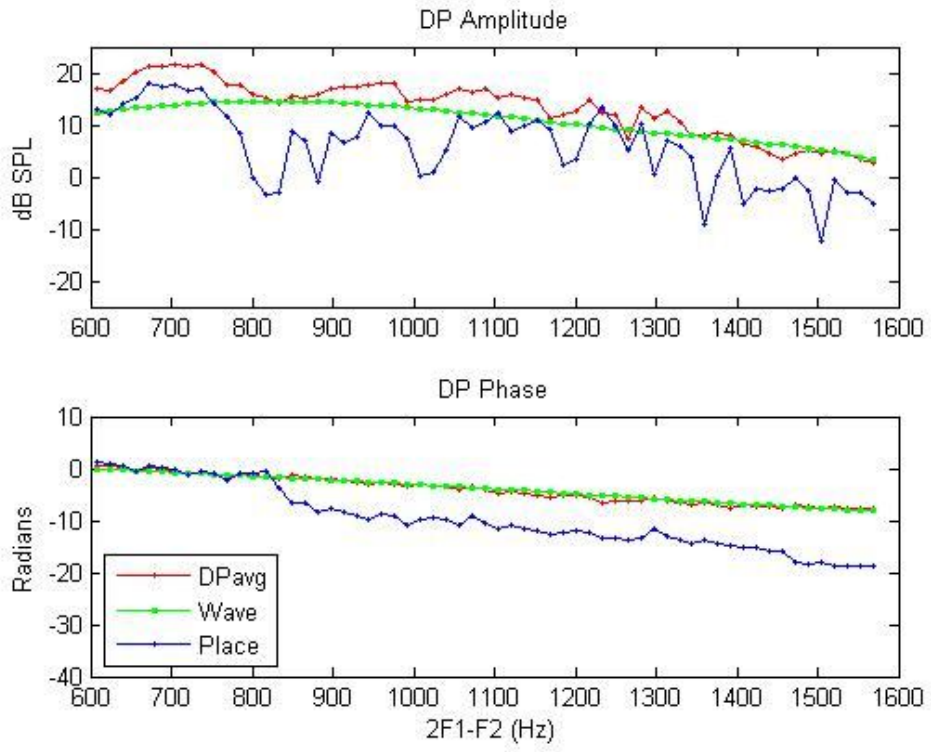

Figure 4.4. Example of MATLAB output showing DPOAE and its components. DPavg, Wave and Place refer to DPOAE, wave- and place-fixed components.

Program 3: Averaging

The recorded DPOAE amplitude data after cleaning and amplitudes of wave- and place-fixed component data after separation were averaged across frequency to obtain a single amplitude value for each recording condition for a given participant. The power averaging was carried out according to the following formula. This formula has been previously used for similar purposes by other studies (Parazzini *et al*., 2005b; Wilson and Lutman, 2006). In this chapter, the amplitude of DPOAE refers to the averaged DP amplitude calculated in this way.

$$DP_{average} = 10Log_{10}\left[\left\{\sum_{i=1}^{n} 10^{DP/10}\right\} / n\right]$$

Where DP is the amplitude in dB SPL and *n* is the number of frequencies.

Amplitude of DPavg (composite DPOAE), wave- and place-fixed components, and difference between components was reported in dB SPL while phase gradient of DPavg is reported in degrees/Hz.

4.2.4. Manipulation of body position

DPOAE and tympanogram were recorded in two body positions: sitting and head down. The second position was obtained by using a reclining couch. The participants rested in a supine position while the head with the help of a head rest made an angle of -10^0 with the horizontal plane. The angle was measured using a protractor with a permissible error of $\pm 2^0$. An arrangement of this manipulation is depicted in Figure 4.5.

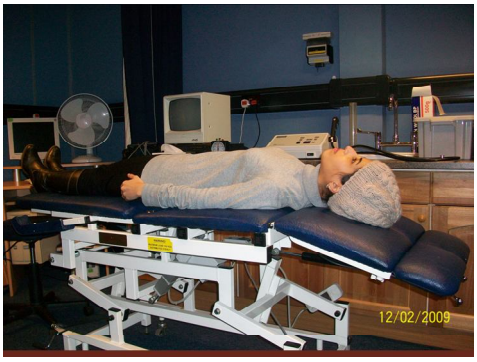

Figure 4.5. A participant resting on a reclining couch for DPOAE testing.

4.2.5. Procedure

The experimental steps for conducting this study are described as follows. The entire experiment took approximately 70-90 minutes. A pilot study with two participants was conducted to ensure that the procedure runs through smoothly.

- The subjects were selected for the experiment following otoscopy, pure-tone audiometry, completion of basic health questions related to ear and hearing disorders, and tympanometry. Each participant signed the consent form prior to the beginning of any experimental procedures.
- Once the subject met inclusion criteria, data collection commenced, including two DPOAE measurements and tympanometry trials recorded for each body position. The manipulation of the body position was performed in counterbalanced order to minimise order effects. The measurements required a refitting of the probe between different positions and occasionally between trials. Tympanometry was always performed prior to DPOAE measurements in order to monitor middle ear pressure. The measurements in two different body positions were separated by a break of at least 5 minutes.

4.2.6. Statistical methods

The tympanometry and DPOAE data were examined for normal distribution using the Kolmogorov-Smirnoff (K-S) test. The K-S test suggested that the raw data were normally distributed. Appendix 4.2 shows a few representative histograms. This meant that parametric statistics could be applied meaningfully. Repeated measures analyses of variance (RM-ANOVA) were performed separately on amplitudes and phase gradient data to examine if there were any significant effects of (i) trials or (iii) body position. Greenhouse-Geisser correction was applied for sphericity where required and Bonferroni adjustment was selected for multiple comparisons. The statistical package used was SPSS for Windows version 15.0. The basic level of significance was always set at 0.05. The measurement parameters were (i) amplitude of DPavg, (ii) amplitude of wave-fixed components, (iii) amplitude of place-fixed components, and (iii) phase gradient of DPOAE (or DPavg).

4.2.7. Results

The mean middle ear pressure was -6.6 daPa (SD=12.1) in sitting position and 7.3 daPa (SD=10.5) in head-down position. The mean increase in middle ear pressure in head-down position was 14 daPa (SD=8.7). The mean static acoustic admittance in sitting and head-down position were 0.89 cm^3 (SD=1.4) and 0.56 cm^3 (SD=0.4) respectively. The values of these measures fall within the ISVR normative data[6], meaning that the middle ear mechanisms were within normal range during DPOAE measurements.

The DPOAE from any given data point for all subjects was accepted if the emission level was more than the noise floor at least by 6 dB. The repeatability of the data was examined by Bland and Altman plots in addition to RM-ANOVA. Bland and Altman plots show the difference between two trials as a function of mean of two trials. In general, these plots show good repeatability of the present data. Figures 4.6, 4.7, 4.8 and 4.9, show the Bland and Altman plots for amplitudes of DPavg, wave- and place-fixed components, and phase gradient of DPavg respectively.

[6] The normative data used at the ISVR for middle ear pressure are -100 to $+50$ daPa and 0.3 to 1.8 cm^3 for static admittance.

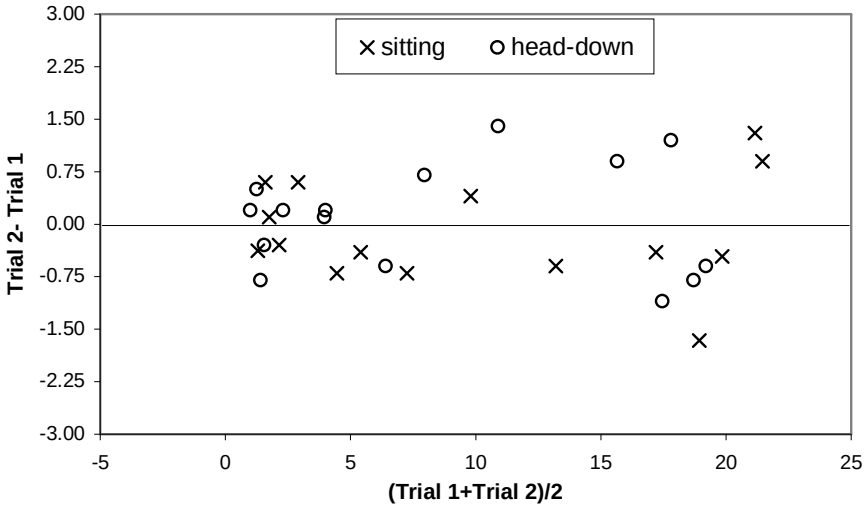

Figure 4.6. Bland and Altman plots for DPavg amplitude (dB SPL).

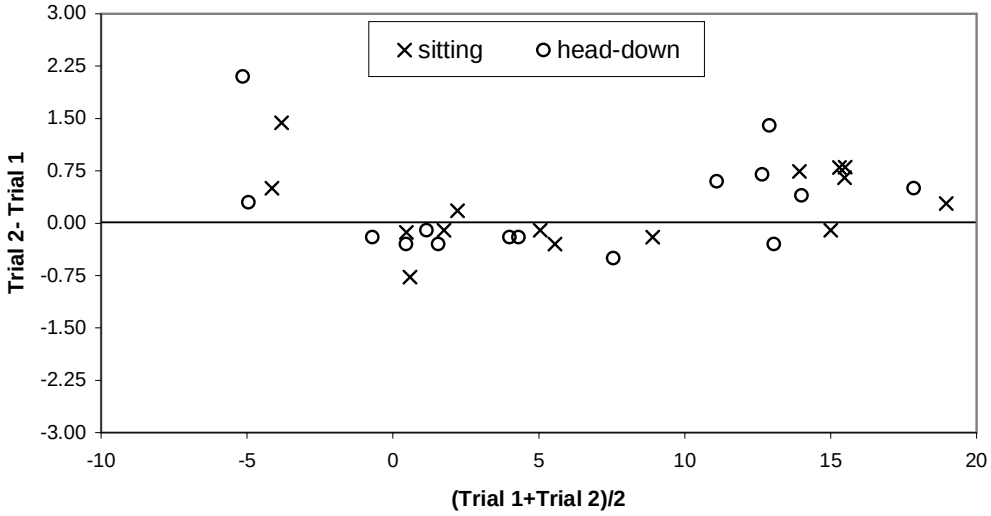

Figure 4.7. Bland and Altman plots for wave-fixed component amplitude (dB SPL).

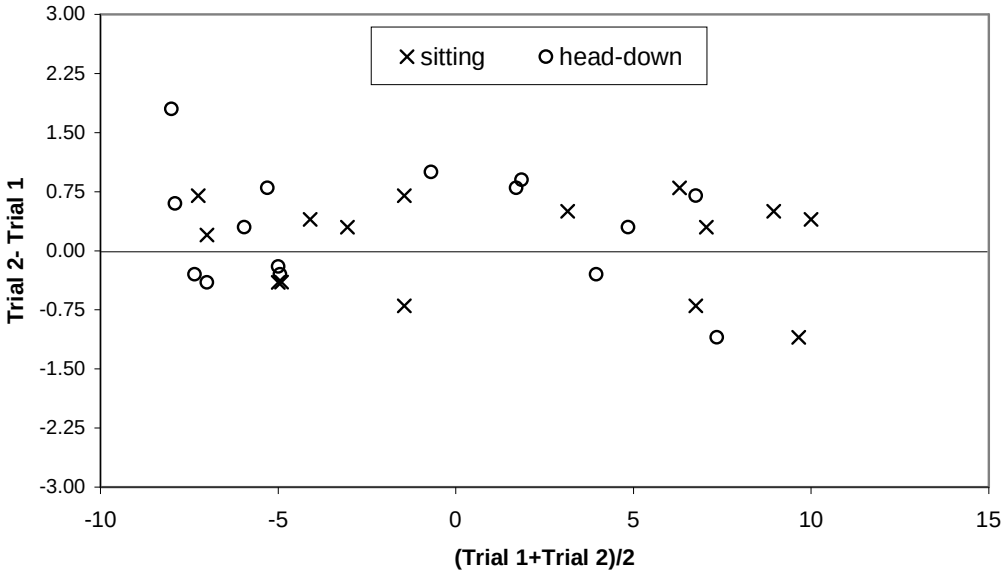

Figure 4.8. Bland and Altman plots for place-fixed component amplitude (dB SPL).

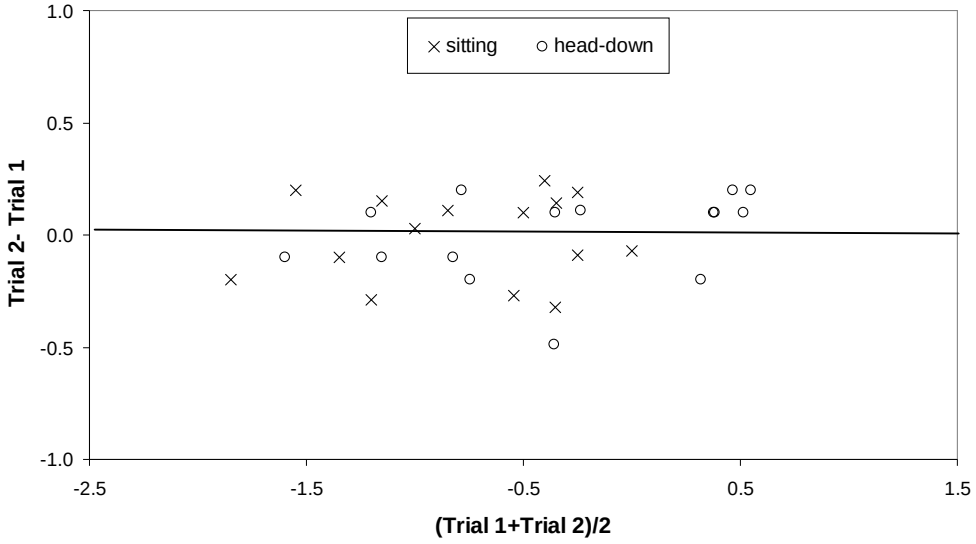

Figure 4.9. Bland and Altman plots for phase gradient (degrees/Hz).

An example of amplitude plot in sitting and head-down position is shown in Figure 4.10. Figure 4.11 shows the phase of DPavg plotted as a function of frequency in the two measurement conditions.

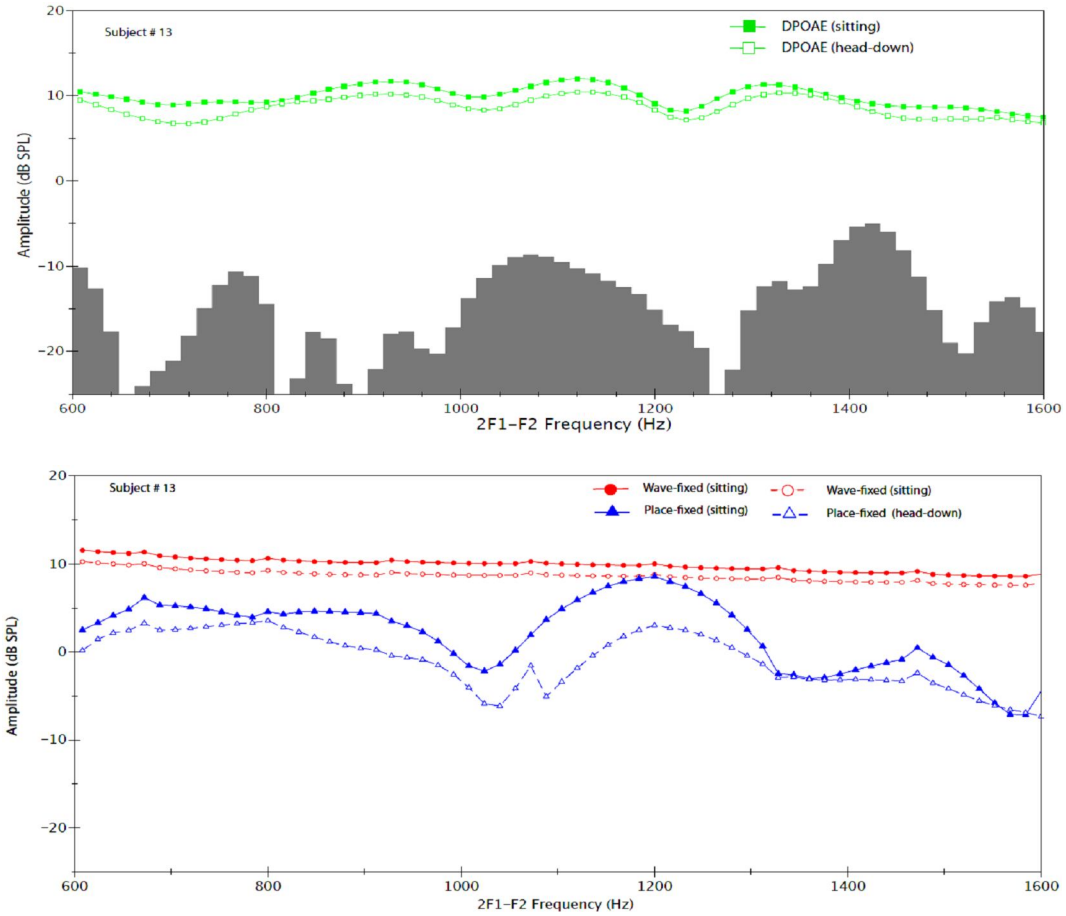

Figure 4.10. Amplitude of the DPavg and the wave- and place-fixed components in sitting and head down positions. Upper panel shows the amplitude of DPavg in sitting position (filled squares) and in head-down position (open squares). The grey shade is the noise floor in sitting position. Lower panel shows the amplitude of the wave- and place-fixed components in sitting and in head down position.

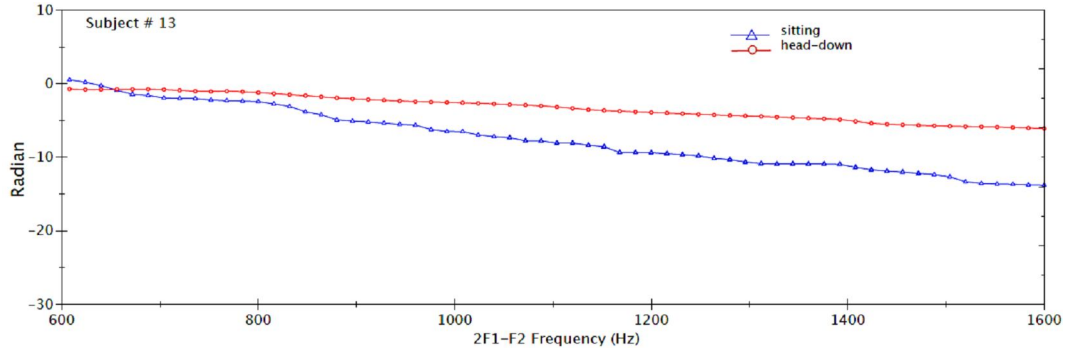

Figure 4.11. An example of Phase plot of the DPavg in sitting and head-down position. The phase gradient value in the head-down position (–0.34 degrees/Hz) is higher than that in the sitting position (–0.81 degrees/Hz).

RM-ANOVA found no effect of trial on the amplitudes but a significant effect of body position on amplitudes of DPavg ($p<0.001$), wave- ($p<0.001$) and place-fixed components ($p<0.001$). The lack of effect trial does not necessarily mean that the results from two trails are same. The amplitudes of all the types DPOAE tend to be reduced in the head-down position relative to sitting position. The mean reduction in amplitude of DPavg from sitting to head-down was 1.3 dB, while the mean differences between trail 1 and trail 2 in sitting and head down position were 0.11 and 0.08 dB respectively. The measures from the two trails were averaged to obtain a single value for the amplitudes and phase gradients. Figure 4.12 shows the mean amplitude of DPavg, wave- and place-fixed components and difference between wave- and place-fixed components in sitting and head-down position. RM-ANOVA of amplitude (DPOAE × body position) was applied to analyze the effect of body position on DPOAE components (wave- and place-fixed). There was a main effect of type of DPOAE ($p<0.001$) and body position ($p<0.001$) and an interaction between the two ($p<0.01$). It is evident from the Figure 4.12 that the amplitudes of wave-fixed components are higher than those of place-fixed components; also, the amplitudes are higher in the sitting position compared to head-down position.

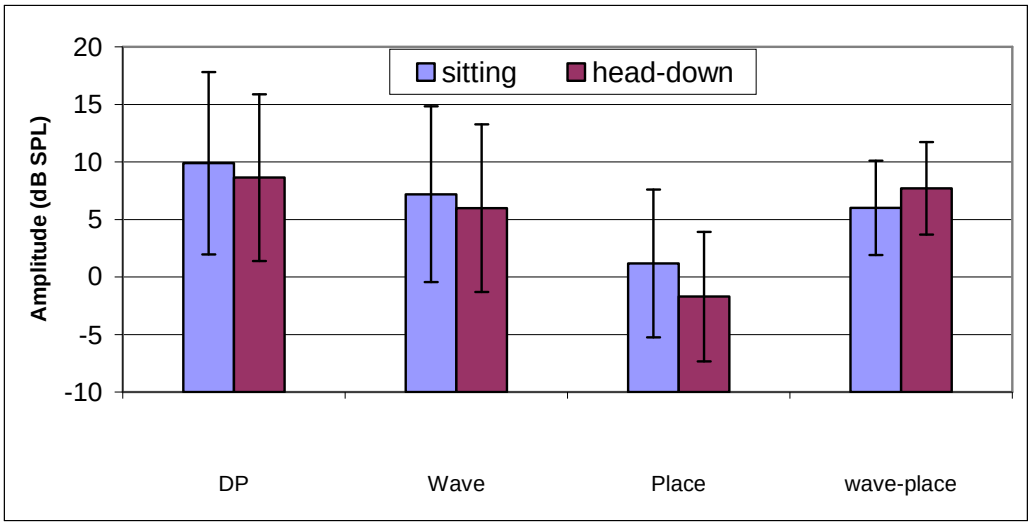

Figure 4.12. Mean amplitudes of various types of emissions. The error bars indicate ±2 SD. DP, Wave, Place, wave-place respectively refer to the DPavg, wave- and place-fixed components and the difference between two components.

Previous analyses showed a difference between DPOAE component amplitudes. The effect of body position on this difference between components was tested by calculating amplitude difference (wave-fixed minus place-fixed). This difference provides information on the relative amplitudes of each component for the DPOAE from a given subject. If the difference is less, then it means that the two components are close to each other in amplitude and conversely, if the difference is more, one of the components is higher in amplitude than the other. The amplitude difference between the two components was calculated and tested with RM-ANOVA. Results showed an effect of body position ($F = 29.8$; $p<0.001$) on the component difference. The head-down position increased the amplitude difference between components (Figure 4.12). As further elucidated in the same figure, the increased component difference was a result of the place-fixed component being reduced more than the wave-fixed component. Change of body position from sitting to head-down produced a mean reduction in the amplitude of the wave-fixed component by 1.2 dB compared to 2.9 dB for the place-fixed component.

Because in the 2F1–F2 DPOAE, the amplitude of the wave-fixed components is higher than that of the place-fixed components, the comparison of the change of DPOAE components in dB scale could be possibly distorted due to the logarithmic and compressive nature of the dB scale. Thus, the amplitude of wave- and place-fixed components were also analysed in the linear scale to examine the effects of body position on the wave- and place-fixed components. The amplitude of DPavg was not analysed in linear scale because it has similar amplitude as the wave-fixed components and also, because the goal of this analysis was to define the relative changes in wave- and place-fixed components due to change in body position. The amplitude of the wave- and place-fixed components for each participant was converted into linear scale (re; 20 μPa) according to the following formula.

$$P(\mu\text{Pa}) = 10^{(\frac{dB}{20})} \times P_{ref}$$

Where, P(μPa) is the DPOAE amplitude in linear scale, dB is the DPOAE amplitude in dB SPL and P_{ref} is the reference (=20 μPa). The change in amplitude for a given component was computed by subtracting the amplitude in head-down position from that in the sitting position. Figure 4.13 presents the mean amplitude (μPa) difference between

sitting and head-down position for wave- and place-fixed components. A paired sample *t*-test revealed that the change in amplitude of the wave- and place-fixed components are significantly different ($p<0.01$). The change in body position induces significantly higher change in the place-fixed component (mean=13.4 μPa) than in the wave-fixed component (=9.8 μPa). This finding confirm with the analysis in dB scale.

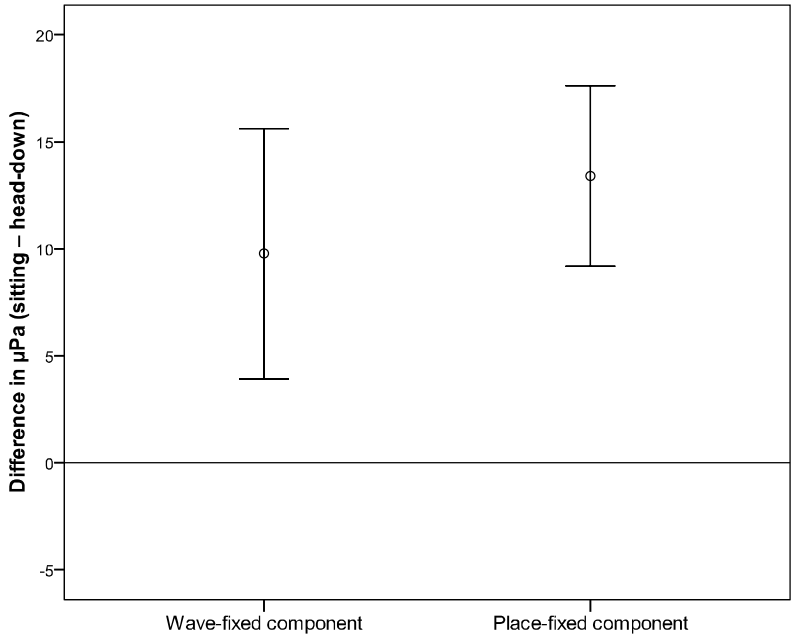

Figure 4.13. Mean amplitude change (sitting – head-down) in the wave- and place-fixed components. Error bars indicate 95% confidence intervals of the mean.

The phase gradient of the DPavg was analysed to interpret (or gather indirect evidence on) the dominance of the component (wave- or place-fixed) in 2F1–F2 DPOAE. Knight and Kemp (1999) suggested a phase gradient of 2 degrees/Hz or more (at F2/F1 ratio=1.2) is associated with a place-fixed dominant DPOAE. For the present purposes, the DPOAE was considered as wave-fixed dominant if the phase gradient of the DPavg is less than 1.5 degrees/Hz. The aim of this analysis was to define the changes (if any) in phase gradient of DPavg in order to determine the dominance of the component, hence, the phase gradient of individual DPOAE components were not assessed. RM-ANOVA showed significant effect of body position on the phase gradient of the DPavg ($p<0.001$) but no effect of trail ($p>0.05$). The mean phase gradient of DPavg in the head-down position (–0.31 degrees/Hz) is higher than that in the sitting position (–0.77 degrees/Hz). This suggests that change in body position from sitting to head-down

caused an increase in phase gradient of the DPavg (mean change= 0.46 degrees/Hz). Figure 4.14 shows the mean phase gradient of DPavg in sitting and head down position and the difference (sitting – head-down).

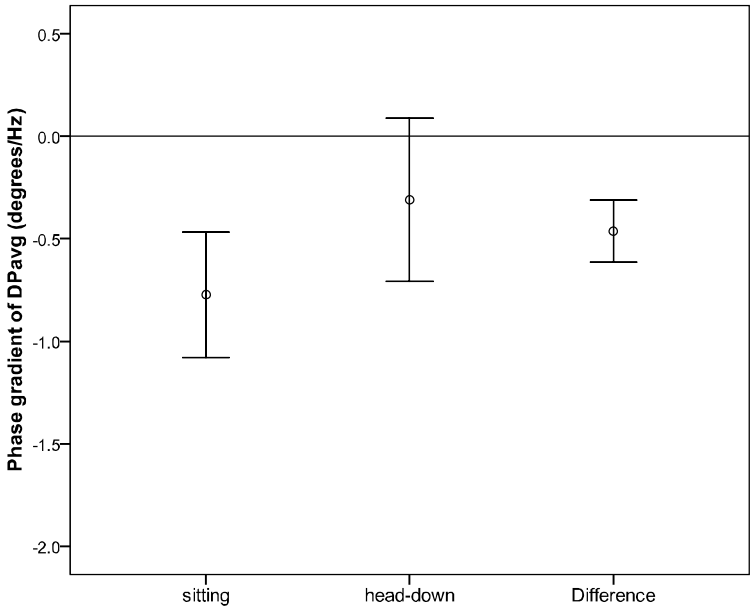

Figure 4.14. Mean phase gradient of DPavg in sitting, head-down position and difference (sitting – head-down). The error bars indicate 95% confidence intervals of mean.

4.2.8. Discussion

DPOAE amplitude and phase change is an obvious effect of body position, thought to be mediated via changes in ICP. There appear to be no previous reports that explore changes in the wave- and place-fixed components of DPOAE and phase gradient due to change in ICP. The separated components (wave- and place-fixed) provide important insight into mechanism of generation of DPOAE. Therefore, the objective of this experiment was to determine how the components of DPOAE change due to change in body position from sitting to head-down position. The middle ear pressure was monitored via tympanometry. The summaries of main findings are:

1. The amplitudes of DPavg, wave- and place-fixed components and phase gradient are stable with repeated measurements.

2. The amplitudes of DPavg, wave- and place-fixed components are reduced in head-down position compared to the sitting position, while the phase gradient increased in the head-down position.
3. The amplitude of wave-fixed components are higher than those of place-fixed components in both sitting and head-down positions, meaning that the dominance of wave-fixed components in 2F1–F2 DPOAE does not disappear with change in body position.
4. The wave- vs. place-fixed component difference is greater in the head-down position compared to the sitting position and this is mainly due to significantly greater reduction of place-fixed components.

The observed changes in DPOAE amplitudes and phase gradient due to change in body position can be attributed to a mixture of several factors: (i) changes in middle ear transmission and/or (ii) changes in cochlear mechanisms due to changes in ICP. Sound transmission through the middle ear is affected due to change in middle ear pressure leading to changes in DPOAE amplitudes in the low-mid frequencies (Huttenbrink, 1998). Alterations in sound transmission due to middle ear pressure changes are thought to originate from the stiffness of the middle ear (stiffness of the tympanic membrane and annular ligament). The middle ear system was thus monitored via tympanometry acknowledging that changes in middle ear pressure, ICP or body position could potentially influence middle ear sound transmission in both directions as needed to record OAE. The ICP in positions similar to head-down has been found to be higher relative to sitting position (Chapman *et al*., 1990).

Although the middle ear pressure measurements were not the primary goal of the present study it is important to eliminate the contributions of middle ear transmission in an attempt to single out changes in DPOAE due to variations in ICP induced by body position. The tympanometry in the present experiment provide sufficient resolution to determine the effect of body position on changes in middle-ear pressure. The mean increase in middle-ear pressure was found to be 14 daPa from sitting to head-down position, while the mean change in static acoustic admittance was 0.33 cm^3. Previous studies along similar lines (Gaihede and Kjaer, 1998; Knight and Eccles, 1991; Tideholm *et al*., 1999) have found that normal hearing participants show no change to an increase of 22 daPa on middle-ear pressure between upright and supine positions. However, there

is no report of changes in middle ear pressure for the body positions of sitting (90°) and $-10°$ (head-down). Similarly, the effects of middle-ear pressure changes of less than ±50 daPa on amplitudes of DPOAE have not been reported. Hauser *et al*., (1993) reported a decrease in DPOAE amplitudes that correspond to middle ear pressure changes in steps of 200 daPa, while Plinkert *et al*. (1999) found mean reductions of about 6 dB in the amplitudes DPOAE at 1000 Hz when the middle ear pressure is reduced from 0 to -100 daPa. These finding cannot be directly applied in the present context because the majority of the DPOAE measurements in the present study have middle ear pressures that vary by a mean of +14 daPa from the sitting position.

The amplitudes of DPOAE and its components decreased as the body position changed from sitting (90°) to head-down (–10°), presumably induced by changes in ICP. This reduction in amplitude due to increase in ICP is in agreement with previous studies. Büki *et al*. (1996), using a nearly similar manipulation of body position, found a frequency dependent effect of the order of 1 dB when middle ear pressure was fixed. However, they have analysed the overall amplitude of the DPOAE (i.e., DPavg in the present study).

The present study additionally measured the changes in the amplitudes of DPOAE components. Separate component measurements are novel techniques that provide a detailed insight of cochlear mechanisms involved in the generation of DPOAE. Although, it is yet to be studied, in principle, several pathological and non-pathological factors may affect one component more or less than the other, for instance suppression effects (described in Chapter 5). Changes in body position induced differential effects on the components of DPOAE, with place-fixed component amplitudes reduced significantly more than wave-fixed components. This indicates that reflection sources were reduced more compared to distortion sources due to change in body position. While this finding cannot be directly compared with others, as there are no studies of this type, the nearest comparison is that auditory threshold microstructure measured psychophysically was less pronounced when the body position was tilted from sitting position and almost disappeared in a horizontal position (Wilson, 1980). This is consistent with the present findings of greater reduction of place-fixed components compared to wave-fixed components in the head-down position. This is primarily because reflection sources are responsible for much of the microstructure in DPOAE

(Talmadge *et al*., 1998). Also, because reflection components tend to travel further towards the apical part of the basilar membrane before they are reflected to contribute to the generation of composite DPOAE, they are thus exposed to regions where the effects of ICP are more prominent (in low-mid frequencies). This is also possibly the reason for increase in phase gradient of the DPOAE in the head-down position. The present results cannot be compared with the phase shift results by Büki *et al* (2000) due to inherent differences in phase measurement techniques and goals. In the present context, phase gradient is interpreted to determine the dominance of a given component in the composite DPOAE. The greater reduction of place-fixed components due to change in body position may be a result of middle ear pressure changes, however, such an effect of middle ear has not been reported previously.

The mean increase in the phase gradient was 0.46 degrees/Hz from sitting to head-down position. The numerical value of this change is high and had this change been in the other direction (to make a phase gradient of 2 degrees/Hz or more), the contribution of place-fixed components in DPOAE would have increased. However, as predicted from the phase gradient measurements and also from component amplitude difference analysis, the dominance of wave-fixed components in the 2F1–F2 DPOAE remains unchanged regardless of reduction in amplitudes induced by changes in body position.

The primary objective of this study was to investigate the changes in components of DPOAE. As expected, DPOAE amplitudes changed with the manipulation of body position presumably due to ICP. However, the findings presented here also highlight the change in phase gradient and differential change in the wave-and place-fixed components due to change in body position. Specifically, place-fixed components are more affected due to change in body position than wave-fixed components. DPOAE phase gradient appears to be increased by changing body position from sitting to head-down implying that the dominance of wave-fixed components in the 2F1–F2 DPOAE remains unchanged.

Future work in determining sensitivity of DPOAE components to ICP changes might also equalize ear-canal and middle-ear pressures in conjunction with DPOAE measurements. Such an approach would require a single probe that would allow measurement of both DPOAE and tympanometry simultaneously. Also, inclusion of a

wide range of frequency ratios and measurement of 2F2–F1 DPOAE might be interesting as place-fixed components are dominant in 2F2–F1 DPOAE.

CHAPTER 5

DPOAE–BASED MEASUREMENT OF THE EFFERENT EFFECTS

5.1. Introduction

This chapter examines the changes in components of DPOAE when cochlear functioning is manipulated using contralateral acoustic stimulation.

It is well-known that MOC modulates cochlear functioning. However, it is unclear if the interaction of MOC with cochlear function alters the generation mechanism of DPOAE (wave- or place-fixed mechanisms). Cumulatively, several authors (Giraud *et al*., 1997b; Kujawa and Liberman, 2001; Müller *et al*, 2005; Wagner *et al*, 2007; Williams and Brown, 1997; Zhang, Boettcher and Sun, 2007) have speculated that the effect of CAS on DPOAE is determined by the interaction of two components (wave- and place-fixed components). Stimulation of MOC may differentially influence each of these components and hence the amount of suppression measured via DPOAE in the ear canal. Since the place-fixed components depend on the prior generation of wave-fixed components it is possible that the effects of CAS on wave-fixed components must have consequential effects on place-fixed components; however, CAS might or might not have any further effects on place-fixed components. While such a hypothesis is conceivable because DPOAE are generated by different mechanisms and at different sites along the basilar membrane, this hypothesis is yet to be tested.

The main objective of this experiment was to investigate the effects of CAS on the amplitude of wave- and place-fixed components. Effects of CAS on the phase gradient of DPOAE were also studied to gather additional evidence to examine the main findings from wave- and place-fixed components. More specifically, experiment was designed to answer the following research questions: (i) does CAS change dominance of the wave-fixed components in 2F1–F2 DPOAE, (ii) what is the effect of CAS on the amplitudes of wave- and place-fixed components of 2F1–F2 DPOAE, and (iii) does phase gradient of 2F1–F2 DPOAE change due to CAS. The working hypothesis was that MOC (activated by CAS) may differentially influence each of these components and hence the amount of suppression measured via DPOAE in the ear canal.

5.2. Method

5.2.1. Participants

Fourteen healthy young adults (19-31 years of age) without any evidence of hearing or ear disorder, corresponding to the ISO definition of otologically normal participated in this study. Acceptance as a participant was based on normal results on otoscopy, hearing thresholds less than 15 dB HL as measured by air- (0.5, 1, 2, 3, 4, 6, 8 kHz) and bone-conduction (0.5, 1, 2 kHz) pure-tone audiometry, normal tympanometry, and a simple screening questionnaire concerning medical and neuro-otological history. All the participants had thresholds below 15 dB HL at all frequencies and normal tympanograms. The ear with better pure tone thresholds was tested - or the one with smaller SOAE if thresholds were the same. All the participants were students of the University of Southampton. A consent form was signed by each participant after the nature of experimental procedure was explained. This study was approved by the Institute of Sound and Vibration Research (ISVR) Human Experimentation Safety and Ethics Committee. All the measurements were carried out in an audiometric booth, which complied with ISO 8253-1 for the measurement of hearing threshold level down to 0 dB HL.

5.2.2. Instrumentation and calibration

The instrumentation for DPOAE has been described in a previous chapter (Chapter 3).

5.2.3. DPOAE paradigm

DPOAE were measured using custom laboratory apparatus described previously. DP-grams were recorded and plotted as a function of F2 for a range of frequencies from 1000- 6000 Hz approximately. The DP sweeps were collected with fixed frequency ratio (F2/F1= 1.22) while F2 was increased in 16 Hz step size. The levels used were L1=60 dB, L2=50 dB. An example of DPOAE recording from a participant in this study is shown in Figure 5.1.

To obtain separate estimates of wave and place-fixed components the DP data were processed using a similar Matlab program adjusted for the F2 frequency range (as described in Chapter 4). Amplitude of DPavg (composite DPOAE), wave- and place-

fixed components, and difference between components was reported in dB while phase gradient was reported in degrees/Hz.

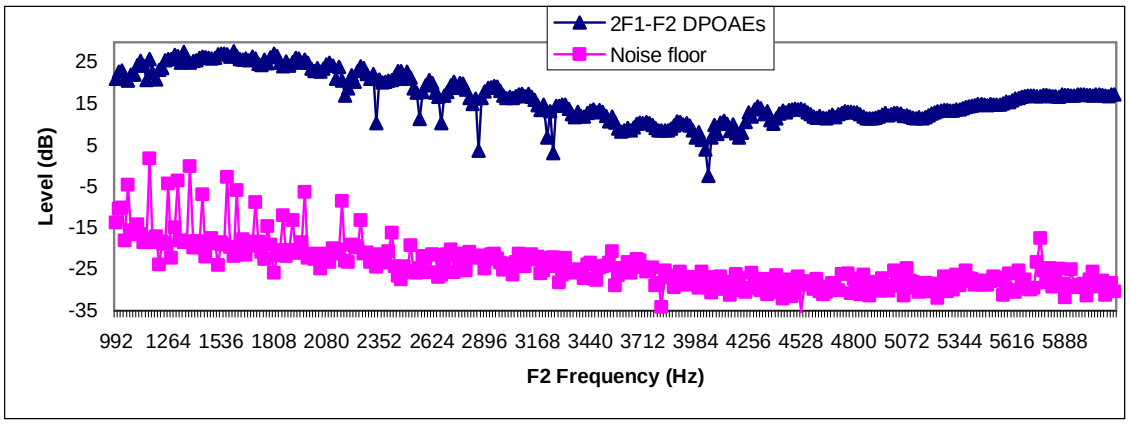

Figure 5.1. An example of raw DPOAE recordings from a participant. The top blue curve (filled triangles) is the level of 2F1–F2 DPOAE and the bottom pink curve (filled squares) are the corresponding noise floor.

A representative example of time-domain representation of the DPOAE is shown in Figure 5.2. A typical example of the program output (plots of the phases and amplitudes of both the wave-fixed and place-fixed components) in the frequency domain as a function of the DP frequency (2F1–F2) is shown in Figure 5.3.

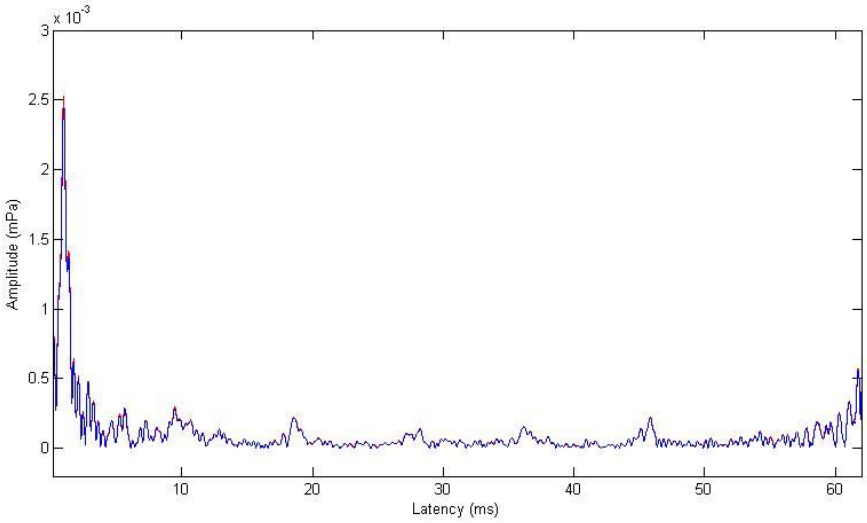

Figure 5.2. An example of time- domain representation of DPOAE after IFFT.

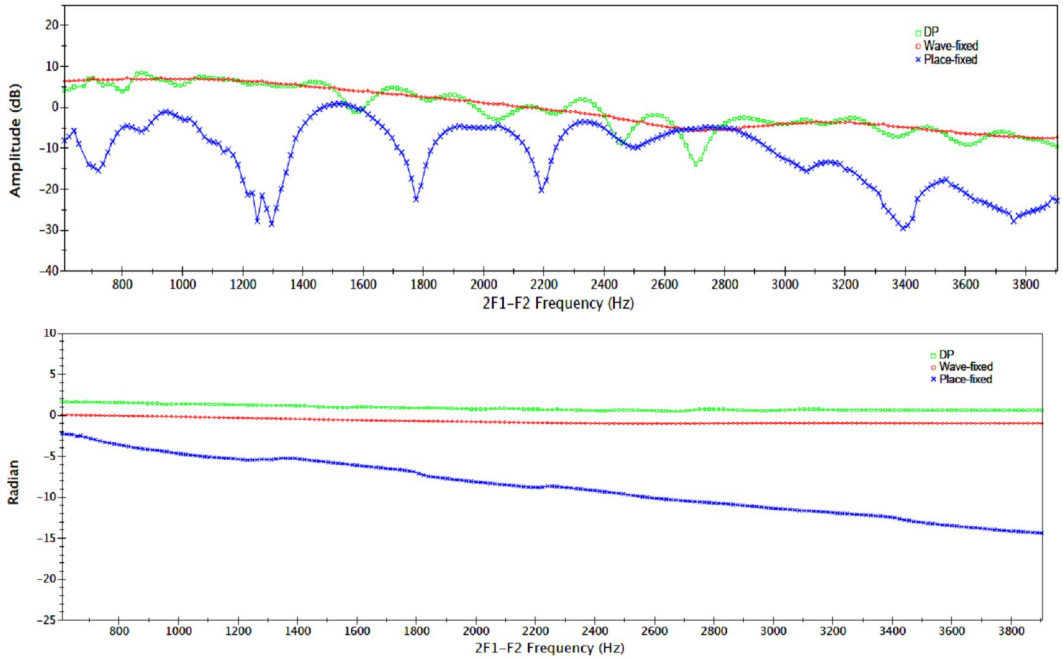

Figure 5.3. An example of amplitude and phase plots after separation of wave- and place-fixed components. DP, Wave, Place refers to the DPOAE after processing, wave- and place-fixed components.

5.2.5. Suppression measurements

DPOAE were measured while the contralateral ear was presented with broad-band noise (BBN) at 35 dB SL via an Etymotic ER-3A insert earphone generated by an audiometer. For the purposes of contralateral stimulation, the threshold of audibility to BBN was also measured according to the same procedure as used for pure-tone audiometry. Ipsilateral acoustic reflex thresholds (ART) were measured to ensure that the level of contralateral acoustic stimulation was insufficient to evoke acoustic reflexes. Ipsilateral ART for BBN were established by an ascending-descending 1-dB bracketing approach. ART was defined as the minimum SPL of BBN at which a reduction of not less than 0.03 ml in middle ear admittance could be measured at least 50% of the time.

The DPOAE measurements with and without contralateral acoustic stimulation were repeated twice in the same session. Each suppression measurement consisted of initial DPOAE measurements without noise followed by DPOAE measurements with contralateral noise. The probe was refitted for the second set of measurements. Suppression was computed by subtracting the amplitude of the DPOAE in contralateral stimulation from that of the DPOAE in the absence of contralateral stimulation. The

suppression of DPOAE amplitude, and amplitudes of wave-and place fixed components was expressed in dB. For example, suppression for DPOAE is the difference in amplitudes of DPOAE between no noise and with contralateral noise conditions. The phase gradient[7] of DPOAE (computation method described in Chapter 4) without and with contralateral stimulation was expressed in terms of degrees/Hz. For simplicity and consistency throughout this chapter CS_DP, CS_wave, CS_place and CS_phase respectively, refer to the suppression of amplitudes of DPOAE, wave- and place-fixed components, and phase gradient, respectively.

5.2.6. Experimental protocol

Each participant was accepted after otoscopy, pure tone audiometry and tympanometry. The thresholds for BBN and ART were measured. Two sets of suppression measurements were carried out. The total test duration was approximately 2 hours. A restricted range of primary levels and frequency ratios was tested as the aim was to complete the testing in a day for each participant.

5.2.7. Statistical analyses

The Kolmogorov-Smirnoff (K-S) test was performed on the DP suppression, wave-fixed suppression, place-fixed suppression data and phase gradient data. The K-S test suggested that the raw data were normally distributed. Representative histograms are presented in Appendix 5.1. This meant that parametric statistics could be applied meaningfully. Repeated measures of analyses of variance (RM-ANOVA) were performed separately on amplitude and phase gradient data to examine if there were any significant effects of (i) repetitions/trials or (iii) CAS. A separate RM-ANOVA was used to compare the amplitudes of wave- and place-fixed components in noise and no-noise conditions. RM-ANOVA focused on within-subject variations rather than the differences between participants. The within-subject factors were repetitions/trials (trial-1 and trial-2) and CAS (with and without noise). The data from two trails were averaged to get a single value in order to enhance the repeatability. Another RM-ANOVA was performed to see

[7] Recall that in Chapter 4, a single best fit line across the test frequencies was applied to the composite DP phase data to compute the phase gradient. For the present experiment, the phase gradient of composite DP was calculated across each octave (and averaged) in two participants; however, pilot analysis did not show any significant improvement in estimation of the phase gradient of the composite DP. Hence, a single best fit line across all the test frequencies was applied to calculate the phase gradient of the DP.

if there were significant differences between CS_DP, CS_wave and CS_place. Greenhouse-Geisser correction was applied to allow for lack of sphericity where required and Bonferroni adjustment was selected for multiple comparisons. The statistical package used was SPSS for Windows version 15.0, which does not allow performance of post-hoc tests as there was no between subject factor. As a result, the 'simple' contrast reference category was used to analyze if there was any significant effects. The basic level of significance was always set at 0.05.

5.3. Results

5.3.1. Acoustic reflex thresholds

The mean ART for BBN was 82.5 dB SPL (SD= 9.5). The mean level of contralateral noise presented for suppression measurements was 56.9 dB SPL (SD= 4.4). These values confirm that the level of contralateral noise in suppression measurements was well below the ART. An examination of individual data set also confirmed the same. Hence, the measured reductions in DPOAE amplitude may not have been caused by acoustic reflexes of the stapedius muscle and are assumed to be genuine efferent suppression effects.

5.3.2. Presence of DPOAE and components

All fourteen participants had DPOAE present at least 6 dB above the noise floor both without and with contralateral noise. Similarly, every participant's DPOAE could be separated into wave-fixed and place-fixed emissions in each condition (absence and presence of contralateral noise).

5.3.3. Repeatability

The test-retest repeatability of the data was determined by the intra-subject SD on replication. This was computed by dividing the standard deviation of the difference between the DP suppression measures obtained in the two sessions by $\sqrt{2}$. The reason for dividing by $\sqrt{2}$ is because the standard deviation of the difference includes the pooled uncertainty of the two measurements and if each replication has the same uncertainty (intra-subject variance) the difference has double the variance. In this study, repeatability is expressed in term of replication SD calculated in this way. For calculation of

replication SD for suppression of amplitudes of DPOAE, wave- and place-fixed emissions, the CS in the two sessions were compared. But for phase gradient replication SD, the two trials without CAS was compared. Table 5.1 provides a summary of the mean and replication SD of the suppression of DPOAE, wave- and place-fixed emissions and phase gradient of the no-noise trials. Amplitudes are expressed in dB and the phase gradient is expressed in degree/Hz. The SD values are low, which suggest that the all the suppression measurements are stable. Of the amplitude measurements, suppression of place-fixed emissions has poorest repeatability. Hence, the DPOAE, wave- and place-fixed emissions suppression data and phase gradient data from the two measurements (without contralateral stimulation) were averaged to obtain a final value for further statistical analysis purposes.

Table 5.1. Mean and replication SD of DP suppression measurement.

	CS_DP (dB)	CS_wave (dB)	CS_place (dB)	Phase gradient (degree/Hz)
Mean	0.93	0.86	1.12	–0.34
Replication SD	0.29	0.54	0.98	0.25

5.3.4. Effects of CAS

RM-ANOVA revealed (i) no significant effects of session/trials ($p \geq 0.05$), (ii) dominance of wave-fixed components compared to place-fixed components ($p < 0.01$), (iii) significant effects of CAS on amplitudes of DP and its components ($p < 0.01$), with reduction in amplitude due to CAS, but no significant effect of CAS on phase gradient ($p \geq 0.05$), and (iv) no significant difference between CS_DP, CS_wave and CS_place ($p \geq 0.05$).

Components

The wave- and place-fixed components were present in both without and with contralateral noise conditions. An example of DPOAE amplitude plot with and without contralateral noise is presented in Figure 5.4. The mean amplitudes of DPOAE, wave- and place-fixed emissions with and without contralateral noise are shown in Figure 5.5. In the no-noise condition, there was a predominance of the wave-fixed components over

the place-fixed components, and this dominance is statistically significant ($p<0.01$). With contralateral noise condition, the mean amplitude of wave-fixed components was significantly higher than that of place-fixed components. This means that the dominance of the wave-fixed components remained unchanged with the presentation of contralateral noise.

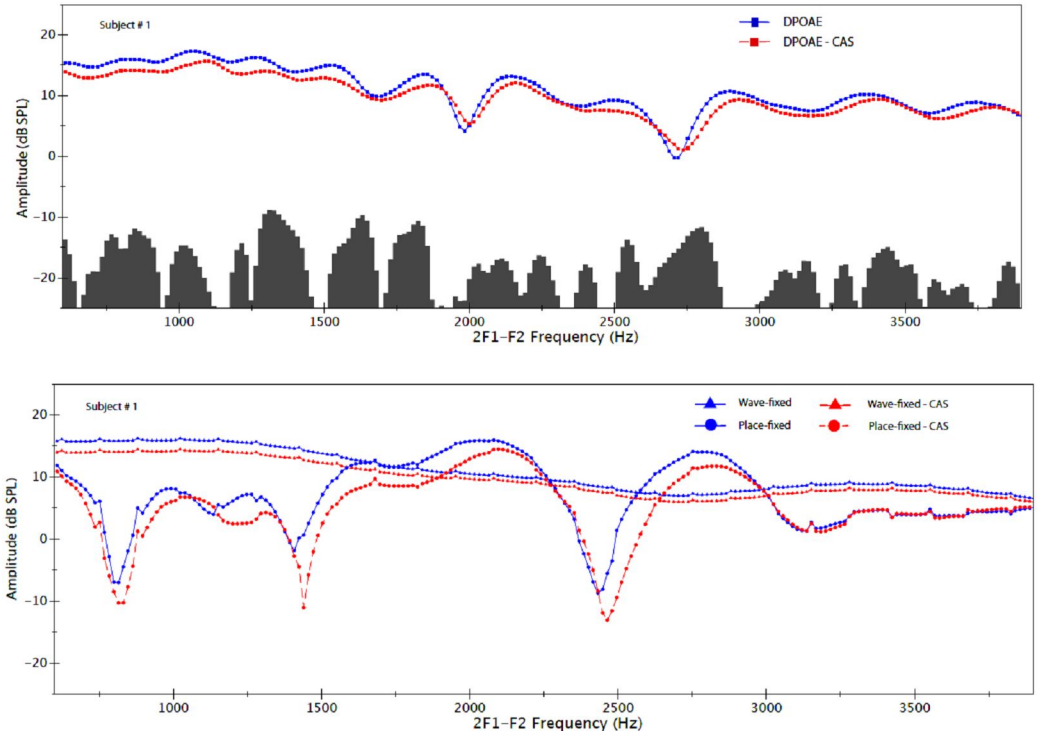

Figure 5.4. Amplitude of DPavg, wave- and place- fixed components with and without contralateral noise. Upper panel shows the DPavg without (blue) and with (red) contralateral noise. The grey shade shows the noise floor. Lower panel shows the amplitude of wave-fixed (triangles) and place-fixed (circles) components without (blue) and with (red) contralateral stimulation.

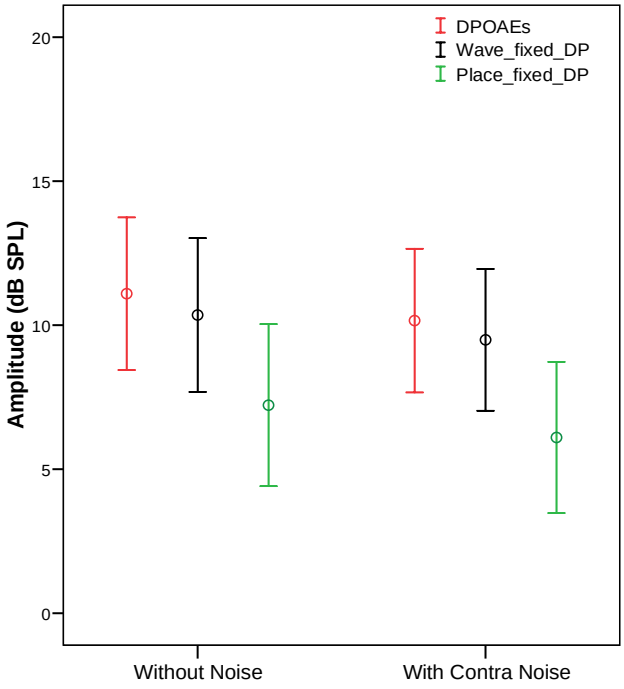

Figure 5.5. DPOAE, wave- and place-fixed emissions with and without contralateral noise. Error bars are 95% confidence intervals of the mean.

Amplitude

The amplitudes of DPOAE, wave- and place-fixed emissions were reduced with the presence of contralateral noise. The mean reductions in amplitudes of DPOAE, wave- and place-fixed emissions were 0.93, 0.86, and 1.12 dB respectively. This reduction in amplitude was statistically significant ($p<0.01$) for DPOAE, wave- and place-fixed emissions. This indicates that contralateral acoustic stimulation induces a significant reduction in the wave- and place-fixed components and thus DPOAE. Figure 5.6 depicts the average amount of suppression of the DPOAE, wave- and place-fixed emissions. It appears that the amount suppression is highest for place-fixed emissions followed by DPOAE and lowest for wave-fixed emissions. The mean difference between CS_wave and CS_place is about 0.26 dB. However, there was no statistically significant difference ($p\geq0.05$) in the amount of suppression between various types of emissions (DPOAE vs. wave-fixed, DPOAE vs. place-fixed, wave- vs. place-fixed). Interestingly, when the CS_wave and CS_place were compared in 12 participants (ignoring participant 11 and 12, who had no suppression, see 5.3.7 section on Individual analysis), suppression of place-

fixed components was found to be marginally significantly higher than that of wave-fixed components ($p=0.052$).

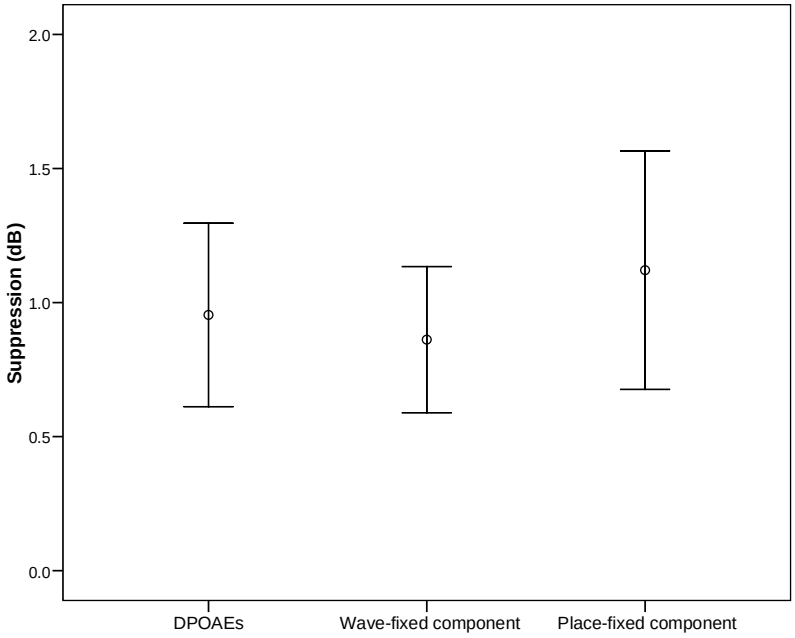

Figure 5.6. Suppression of the DPOAE, wave- and place-fixed emissions. Error bars are 95% confidence intervals of the mean.

Phase gradient

A representative example of phase plot of DPOAE components without and with contralateral noise is shown in Figure 5.7. The mean phase gradients of DPOAE and its components with and without contralateral noise are shown in Figure 5.8. The phase gradient of DPavg value is close to zero and indicates the dominance of wave-fixed components in both no noise and with contralateral noise conditions. As expected, the mean phase gradients of the DPavg (–0.35) and wave-fixed component (–0.23) are similar. Place-fixed component shows a high gradient with a mean slope of –1.2 degrees/Hz. Statistical analysis showed that there was no significant difference in the phase gradient between absence and presence of contralateral noise ($p \geq 0.05$) regardless of DPOAE type. This suggests that contralateral acoustic stimulation has no significant effect on the phase gradient of the composite DPOAE or its components.

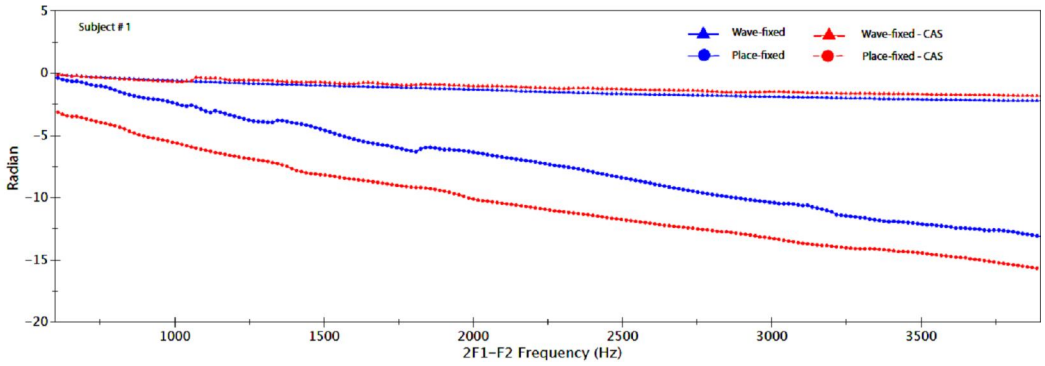

Figure 5.7. An example of phase plot of wave- and place-fixed components as a function of frequency with and without contralateral noise.

A representative example of phase plotted as a function of frequency for wave-fixed (triangles) and place-fixed (circles) components without (blue) and with (red) contralateral stimulation. The phase plot of DPavg (not shown) was similar to that of the wave-fixed component.

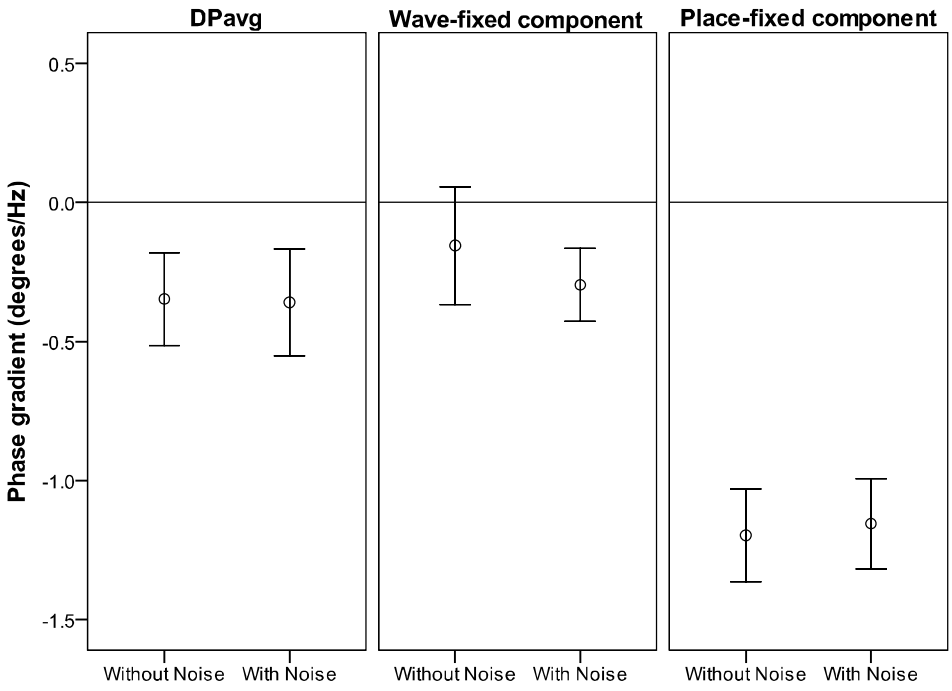

Figure 5.8. Mean phase gradient of DPavg, wave- and place-fixed component without and with contralateral stimulation. Error bars are 95% confidence intervals of the mean.

5.3.5. Relationship between suppression of components

Pearson's product moment correlation coefficient (r) was computed to study the relation between the suppression of various types of emissions. The correlation coefficients from suppression data were calculated for pairs: (i) CS_DP and CS_wave, (ii) CS_DP and CS_place, and (iii) CS_wave and CS_place. The most significant correlation (r= 0.802; p=0.001) was found between the suppression of DPOAE and suppression of wave-fixed emissions, confirms that individuals with greater suppression of DPOAE have also greater suppression of wave-fixed components. CS_DP and CS_place are also significantly related (r= 0.685; p=0.007). A weaker relation was found between CS_wave and CS_place (r=0.605; p=0.022). Scatter plots showing the relationship between suppression of wave-fixed and place-fixed components in Figure 5.9.

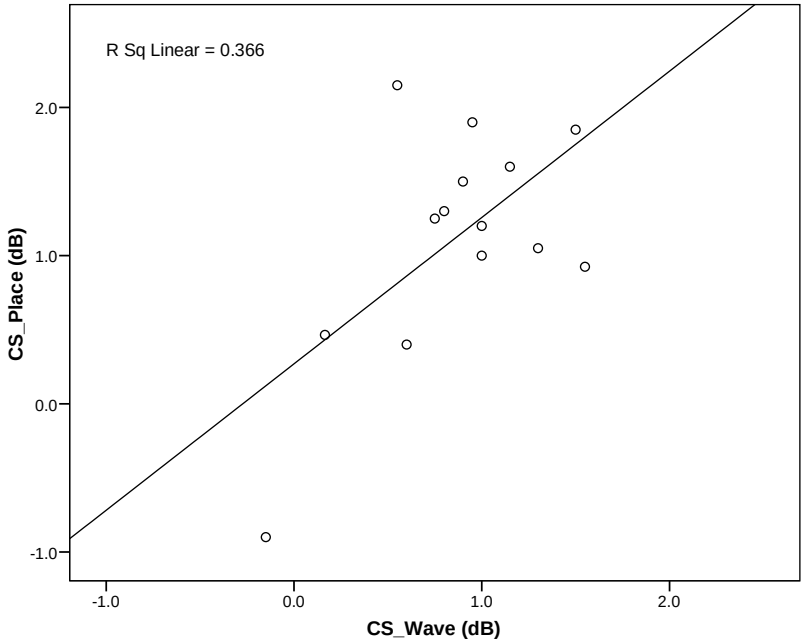

Figure 5.9. Scatter plot showing the relationship between suppression of amplitudes of wave- (CS_wave) and place-fixed (CS_place) components (r^2 linear=0.366).

5.3.6. Analysis of suppression of components in linear scale

In 2F1–F2 DPOAE, the amplitude of the wave-fixed components is higher than that of the place-fixed components. As a result, the comparison of the suppression of DPOAE components in dB scale could be possibly distorted due to the logarithmic and compressive nature of the dB scale. Because the place-fixed components have relatively

lower amplitude than the wave-fixed components, the meaning of 1 dB of suppression would be different for the two different components if examined in linear scale. In other words, depending on the amplitude difference between the components, the wave-fixed components would require less numerical change in dB scale than the place-fixed components to have similar numerical change in linear scale (e.g., μPa). For example, a change in amplitude of the wave-fixed component from 10 to 9 dB (63.25 to 56.37 μPa) corresponds to a suppression of 6.88 μPa in the linear scale. And a change in amplitude of the place-fixed component from 5 to 3.13 dB (35.57 to 28.68 μPa) corresponds to a similar suppression (of 6.89 μPa).

The amplitude of the DPOAE, wave- and place-fixed components for each participant was converted into linear scale (re; 20 μPa) according to the following formula.

$$P(\mu\text{Pa}) = 10^{(\frac{dB}{20})} \times P_{ref}$$

Where, P(μPa) is the DPOAE amplitude in linear scale, dB is the DPOAE amplitude in dB SPL and P_{ref} is the reference (=20 μPa).

The amount of suppression (in linear scale) was then computed by subtracting the amplitude of the DPOAE with contralateral stimulation from that of the DPOAE without contralateral stimulation. The main goal of this analysis was to verify if the suppression of place-fixed components is significantly higher than that of the wave-fixed components. The analysis was conducted in 13 out of the 14 participants (ignoring participant 12, in whom enhancement was seen). A paired sample *t*-test revealed significantly different suppression of the wave-fixed components than that of the place-fixed components ($p<0.01$). Mean suppression of the place-fixed components (11.99 μPa) was higher than that of the wave-fixed components (8.85 μPa). Figure 5.10 shows the mean suppression of DPOAE and its two components in linear scale.

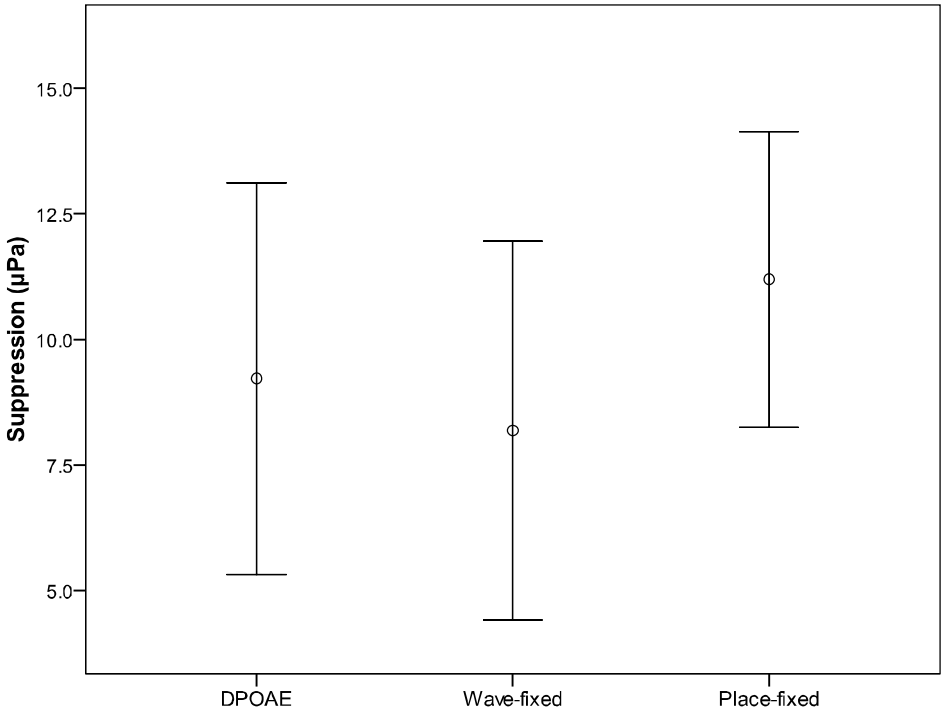

Figure 5.10. Suppression of the DPOAE, wave- and place-fixed components. Error bars indicate 95% confidence intervals of the mean.

5.3.7. Individual analysis

While the group data give a good indication of the results they may not reflect individual differences. The individual analysis is particularly important for suppression measurements which have been found to be variable across participants. The wave- and place-fixed components could be separated in all participants. To determine, if CAS caused suppression in a given participant the amplitudes from all the data points in no noise and contralateral noise conditions from a given set of DPOAE sample were compared using paired *t*-test (p=0.05). The total number of data points per sample ranged from 200-207 depending upon the noise floor of the participant. CAS caused significant suppression in 12 out of 14 participants. Appendix 5.2 provides a summary of the amount of suppression and the phase gradient for each participant from trial-1 and representative example of a DPOAE recording with and without contralateral noise condition. In two participants (participant 11 and 12), no change in the amplitude of composite DPOAE and wave-fixed component was seen while the place-fixed

component in one of these participants (participant 12) increased due to contralateral stimulation. The increase in DPOAE amplitude due to CAS is called as enhancement.

5.4. Discussion

The aim of this study was to examine the effects of CAS on the components and phase of DPOAE. The summary of the main findings is:

(i) Wave-fixed is the dominant component in 2F1–F2 DPOAE and this dominance does not change with CAS.
(ii) CAS has greater effects on place-fixed than wave-fixed components.
(iii) The phase gradient of the composite DPOAE, wave- and place-fixed component does not change with CAS.
(iv) Suppression of amplitude of DPavg is significantly related to the suppression of wave-fixed components.

5.4.1. DPOAE measurement paradigm

While the main objective of this experiment was to investigate the effect of CAS on wave- and place-fixed components of DPOAE, the experimental protocol was chosen to ensure that all the measurements are completed in a single test session. As a result, a single L1/L2 combination and a fixed frequency ratio were selected. The choice of levels of L1 and L2 was based on the report by Kummer *et al*., (2000) to optimize the difference between healthy and impaired cochlear functioning. The range of F2 frequencies from 1000-6000 Hz was tested to gather wider evidence for component separation and suppression. The instrument allows narrowing of the frequency increment size up to 16 Hz. Previous studies on DP component separation have indicated that 2F1–F2 wave-fixed components are more robust at frequency ratio (F2/F1) of 1.22 and place-fixed components are more or less independent of frequency ratio (Knight and Kemp, 2001; Wilson and Lutman, 2006).

5.4.2. Are suppression measurements free from acoustic reflex and other effects?

In suppression measurements, it is important to rule out the involvement of middle ear muscles because the CAS could potentially evoke middle ear muscle reflexes (MEMR)

which may contaminate the measurements. The relative contribution of the MEMR and MOC are species dependent. For example, the suppressive effects are predominantly due to MEMR in rats (Relkin *et al*., 2005) and dominated by MOC in humans (Giruad *et al.,* 1995). Measuring acoustic reflex thresholds is one of the ways to ensure that measurements are free from MEMR effects. In the present experiment, the mean difference between level of ART and contralateral noise was 25.6 dB (range 15-35). This large difference indicates that the level of contralateral acoustic stimulation was insufficient to evoke weak acoustic reflexes, however, very weak MEMR may not be detected by clinical immittance meters. In general, MEMR prevent intense acoustic stimulus from reaching cochlea principally at the frequencies below the resonance frequency of the middle ear, which is around 1200 Hz in adults and the present measurements focused at frequencies above 1 kHz. Furthermore, measurement of the stapedial muscle electromyogram in animals shows similar thresholds to those based on mechanical effects and impedance/admittance change (Counter and Borg, 1979). This suggests that there are no minor effects at lower intensities. Overall, in the present study the possibility of the MEMR occurring during the contralateral acoustic stimulation measurements is very remote, if it cannot be ruled out entirely. In contrast, most of the previous studies on similar lines have attempted to exclude the possibility of MEMR in normal hearing listeners by theoretical arguments rather than actually measuring it in each participant. One of the other effects in suppression measurements that could potentially lead to measurement errors to a lesser extent is masking due to interaural crossover. In this study, interaural crossover due to contralateral sound can be eliminated as the participants had normal hearing thresholds; the insert earphone that was used to deliver the BBN has typical interaural attenuation value of 70 dB, and the presentation level of BBN was rather low (mean 56.9 dB SPL). Although it remains to interpret if the changes are entirely due to MOC, both the terms suppression and MOC are used in this chapter.

5.4.3. Are the data repeatable?

Most studies including the present one have found DPOAE amplitude suppression values to be quite small (around 1 dB). This warrants verification of the repeatability of the data before any conclusions can be drawn. The present data suggest that the suppression and phase gradient measurements are repeatable. It is important to note that

after each set of suppression measurements the probe tip was refitted. Given this, the suppressive effect can be considered as a repeatable parameter for each participant. The repeatability of DP_CS corroborates the findings by Wagner *et al*. (2007). Although there is no study to directly compare the replication SD values for CS_wave, CS_place and phase gradient, a nearest comparison can be made with reliability of phase gradient and wave- and place-fixed components of DPOAE reported by Parazzini *et al*. (2005a and 2005b). The values were a bit higher than the present study. The replication SD values reported by Parazinni *et al*. (2005a and 2005b) for wave- and place-fixed components are 2.25, 1.39 dB respectively and for phase gradient of DPavg is between 0.16- 0.50 degrees/Hz depending upon the F2 frequency, frequency ratio and level. The mean phase gradient of place-fixed component in the present experiment was slightly lower than that of the place-fixed dominant DPOAE (2 degrees/Hz) reported by Knight and Kemp (1999). It might be related to the differences in measurement conditions, Knight and Kemp (1999) provided this value based on DPOAE measurements centred at 2 kHz in one subject.

5.4.4. Which is the dominant component? Does dominance changes with CAS?

The wave- and place-fixed components were present in without and with CAS conditions. In the no-noise condition, as expected there was a predominance of the wave-fixed component over the place-fixed component. This is evident from the significantly higher amplitude of wave-fixed components compared to that of place-fixed components. In addition, the phase gradient values close to zero also indicated the dominance of wave-fixed components. The dominance of wave-fixed components is similar to the results reported by (Knight and Kemp, 2001; Parazzini *et al*., 2005 a, b; Wilson and Lutman, 2006). All of these studies have found a dominance of wave-fixed components at a frequency ratio of 1.22. The consistency in results with previous studies provides a good indication that the findings made regarding suppression measurements are robust and also suggests that the separation program used is reliable.

With CAS, the mean amplitude of wave-fixed components remained significantly higher than that of place-fixed components. This means that the dominance of the wave-fixed components in the 2F1–F2 DPOAE remains unchanged with the CAS. In other words, the MOC reflexes do not change the dominance of wave-fixed components in the 2F1–

F2 DP. While this finding cannot be directly compared with other studies due to non-availability of literature, the phase gradient values close to zero even with CAS do indicate the continued dominance of wave-fixed components. This may mean that the generation mechanisms of 2F1–F2 DPOAE do not change with the activation of MOC via CAS. This may be because CAS suppresses the place-fixed components to a greater extent in dB terms, as a result, the dominance of the wave-fixed component is still maintained in the 2F1–F2 DPOAE; however, this is not the case in at least one participant (participant 12) who had enhancement of the place-fixed components due to CAS. Another speculation is that ipsilateral MOC reflexes possibly interacted in some way to maintain the dominance of the wave-fixed components. Such an assumption stems from the fact that although CAS stimulated the contralateral MOC pathway, the ipsilateral pathways could have been activated by the primary tones (for evoking DPOAE) itself. However, the change in measurement results due to probe tone-elicited MOC activity is unknown. Whatever the reason may be, it appears that MOC helps to maintain the dominance of the wave-fixed components in 2F1–F2 DPOAE in the present scenario.

5.4.5. Which component is suppressed due to CAS?

CAS induced a significant reduction in the amplitudes of DPOAE in 12 out of 14 participants. The mean reductions in amplitudes of DPOAE, wave- and place-fixed emissions were 0.93, 0.86, and 1.12 dB respectively (n=14). The mean suppression of DPOAE is more or less same as previous reports (Bassim *et al*., 2003; Chery-Croze *et al*., 1993; Di Girolamo *et al*., 2001; James *et al*., 2002; Janssen *et al*., 2003; Kim *et al*., 2002; Lisowska *et al*., 2002; Moulin *et al*., 1993; Müller *et al*., 2005; Moulin and Carrier, 1998; Sasaki *et al*., 2000; Sliwinska-Kowalska and Kotylo, 2002; Timpe-Syverson and Decker, 1999; Williams and Brown, 1995; Zhang, Boettcher and Sun, 2007). Recently, Wagner *et al*. (2007) found that MOC effects depend upon the peak or notch of the fine structure and are critically related to the primary tone levels. Several authors (Giraud *et al*., 1997b; Kujawa and Liberman, 2001; Müller *et al*., 2005; Wagner *et al*., 2007; Williams and Brown, 1997; Zhang, Boettcher and Sun, 2007) have argued that the suppression or enhancement of DPOAE due to CAS depends upon the interaction of the two components of DPOAE. The present findings suggest that CAS suppresses both wave- and place-fixed components in majority of the test participants (12/14), and in one

participant (participant 12) place-fixed components were enhanced due to CAS. The reasons for this are unclear, but could be individual variability. The exact reason for enhancement in participant 12 is unknown and possibly related to intra-subject variability in suppression. It suggests that this individual variability in DPOAE suppression cannot be overcome by component separation. Although there is no mean difference in the CS_wave and CS_place (n=14), CAS appears to affect the place-fixed components to a greater extent compared to wave-fixed components, when two participants (who had no suppression) were excluded from the analysis. This clearly demonstrates that combining enhancement and suppression across participants to derive mean values clouds the potential strength of not only suppression of DPOAE but also its components. The presence of enhancement of place-fixed component in one participant indicates that enhancement phenomenon does exist even after separation of components of DPOAE. The reason for this is unclear; however, it may be speculated that enhancement in DPOAE occurs due to lack of suppression of one component that disrupts the destructive interference between two components (the place-fixed components in this case). Because TEOAE arise from predominantly place-fixed mechanisms (Shera and Guinan, 1999), the enhancement of place-fixed components also indicates that enhancement might be seen in TEOAE suppression measurements under equivalent measurement conditions at least in some participants.

The difference in CS_wave and CS_place might mean that in addition to suppression of wave-fixed mechanisms there is some additional suppression of place-fixed components. Williams and Brown (1997) using vector analysis in four subjects provided qualitative insight that CAS may have greater effect on the more delayed component from the DP place. The reasons for higher suppression of place-fixed components is possibly because these components are relatively low-level emissions and are more vulnerable to MOC activity however, this has not been verified in animal experiments. Another possibility is that, as parts of the DP wave travel apically to produce place-fixed emission (Shera and Guinana, 1999), they are more exposed to MOC effects. When the two primary tones are presented to record 2F1–F2 DPOAE, the F1 tone may interfere with the vibration at the F2 place which may alter the operation of the OHCs (which would have been operating in a different way if there was only F2) by reducing the gain to the additional energy due to the F2 tone, and as a result, the MOC action on the cochlear amplifier becomes less effective. The differential suppressive effects of the components of DPOAE might

indicate that place-fixed components are more sensitive to changes in cochlear mechanisms due to stimulation of MOC. This may also explain why the effect of MOC is more pronounced in TEOAE suppression measurements (Berlin *et al*., 1993; Giraud *et al*., 1996; Ryan *et al*., 1991) because TEOAE arise from place-fixed mechanisms. Further, studies on noise induced hearing loss have also indicated that noise induced subtle cochlear changes can be detected early by TEOAE compared to DPOAE (Hall and Lutman, 1999; Plinkert *et al*, 1999; Shupak *et al*, 2007). This evidence supports the argument for vulnerability of place-fixed components.

Correlation analysis showed that suppression of DPOAE is strongly related to the suppression of wave-fixed component and relatively weakly to that of place-fixed components. Thus, in general if wave- and place-fixed components are suppressed then DPOAE is suppressed. The relation between CS_wave and CS_place may mean that the suppression of place-fixed components does have some contributions from suppression of wave-fixed mechanisms. This is consistent with the DPOAE generation model suggested by Shera and Guinan (1999) which explains that the energy from distortion site must be the source of reflection components.

5.4.6. Does CAS change the phase gradient of DPOAE?

It appears that CAS does not have any significant effect on the phase gradient of the composite DPOAE. Also, there was systematic effect of CAS on the phase gradient of the wave- and place-fixed components. The lack of CAS effect on the phase gradient of DPavg is supported by the finding that the dominance of wave-fixed components in 2F1–F2 DPOAE did not change with contralateral stimulation. This is also consistent with findings of no effect of CAS on latency by Relkin *et al*. (2005) in animals and mean group delays by Williams and Brown (1997) in adults, while the minor difference with the findings by Giraud *et al*. (1997b) can be attributed to the test frequencies. Giraud *et al*. (1997b) found that DPOAE latency in normal hearing individuals was shortened at low frequencies (0.8- 2.3 kHz) with CAS. The phase gradient method used in this study has been suggested to be a very sensitive measure to distinguish subtle differences between normal and abnormal cochlear mechanisms (Parazzini *et al*., 2005a). Because wave-fixed components dominate the 2F1–F2 DPOAE and there was no change in the dominance due to CAS so there was no change in phase gradient of the composite DPOAE. It may

be assumed that the effect of CAS on latency/phase of any type of emissions will depend on the dominance of the component. This sort of assumption might also explain the change in latency of TEOAE due to CAS (Berlin *et al*., 1993; Giraud *et al*., 1996; Ryan *et al.,* 1991) considering that place-fixed components dominate TEOAE (Shera and Guinan, 1999).

The lack of effect of CAS on the phase gradient of the wave-fixed component is conceivable because of the assumptions of the separation method. The "wave-fixed" component by definition has near zero phase gradient - that is the assumption and principle of the separation method. The separation method attributes the remainder of the DP by definition to the "place fixed" component and its phase rotates. The phase behaviour is linked to the principles of the separation method rather than providing additional information on cochlear physiology (at least for the wave-fixed component). Regardless of presence or absence of CAS the phase gradient of the wave-fixed component has to be near zero as long as the separation is accurate. The analysis of the phase gradient of the place-fixed component answers the question if CAS produces additional rotations in phase of the reflection sources. The present data seems to suggest that CAS does not produce any change on the phase properties of the place-fixed components. However, it is important to note that currently there is no defined or reported way to compute the phase gradient of the place-fixed components. Because the phase of the place-fixed components varies rapidly with frequency, estimating its gradient might require much sophisticated technique than used in this study. It remains to be answered what is the right way to estimate the gradient of fast-varying phase of the place-fixed components.

5.4.7. Mechanism of DPOAE suppression

Most of the current theories suggest that contralateral suppression or MOC mediated changes in amplitude of DPOAE depend upon the peak or notch of the fine structure and is critically related to the primary tone levels. DPOAE suppression has been related to the interaction of the two components (Giraud *et al*., 1997b; Kujawa and Liberman, 2001; Müller *et al*., 2005; Wagner *et al*., 2007; Williams and Brown, 1997; Zhang, Boettcher and Sun, 2007). The present study extends the understating of mechanism of DPOAE suppression by providing evidence that CAS differentially affects both wave-

and place-fixed components without changing the dominance of the wave- fixed components in 2F1–F2 DPOAE. Compared to wave-fixed components, the place-fixed components are relatively more influenced in dB terms by the MOC activated by CAS in majority of the participants. Measurements of suppression of DPOAE components may provide a better index of changes in cochlear mechanisms due to MOC activity. Enhancement due to CAS might also occur even after component separation. The lack of CAS effect on the phase of composite DPOAE may mean that DPavg may not accurately reflect very subtle changes in delay properties of cochlear mechanisms because of the dominance of the wave-fixed components in the 2F1–F2 DPOAE. It may also mean that MOC activation by contralateral stimulation may not produce any significant change in the way the two components interact to generate composite DPOAE in the ear canal. Future experiment could provide new insight into mechanism of DPOAE suppression if different levels of primaries and CAS are used. Also, by using different F2/F1 ratios, the dominance of wave-fixed component can be altered (Wilson and Lutman, 2006), which may further our knowledge.

CHAPTER 6

ROLE OF EFFERENT AUDITOY SYSTEM ON SPEECH PERCEPTION

6.1. Introduction

This chapter examines the changes in TEOAE when cochlear functioning is manipulated using contralateral acoustic stimulation. It also addresses the functional relevance of such a change.

With the opportunity to measure the performance of the efferent system objectively, non-invasively and possibly behaviourally, recently there has been a revived interest in investigating the functions of this less explored auditory subsystem. Given the inconsistencies in reported psychoacoustic experiments (reviewed in Chapter 2, section 2.4), limited knowledge of the functions of the efferent auditory system, and the interest to identify all the systems and parameters that could potentially contribute to the understanding of the neurophysiologic bases of speech perception in noise, it is important to examine the role of the MOCB systematically for perception of complex signals such as speech in noisy environments in humans.

By relating physiologic measures of MOCB functioning to behavioural measures of speech perception in noise obtained in the same participants, the present study aimed at further investigating the hypothesized relationship between MOCB functioning and speech perception in noise in human participants with normal auditory functioning. More specifically, the principle underlying the study was that, if as suggested by physiological data in animal models, activation of the MOCB leads to improved signal detection in noise, then detrimental effects of background noise on speech perception should be reduced by contralateral acoustic stimulation known to excite MOCB fibres projecting into the test ear. To test this primary hypothesis, we measured SNR scores in background noise, in the absence and presence of contralateral acoustic stimulation. Furthermore, if the change in speech recognition in noise is observed upon contralateral acoustic stimulation, there may exist a quantitative relationship between the change in speech recognition in noise due to contralateral acoustic stimulation and the OAE amplitude suppression by the same contralateral noise. Consequently, the second part of the study tested the relationship between contralaterally induced changes in speech

recognition in noise and transient evoked otoacoustic emissions (TEOAE) measured in the same participants. The underlying principle is not very novel and a few studies using speech stimuli have been conducted on similar lines (see section 2.4). The primary idea of the present study was to verify if the hypothesis can be supported with a different measure of speech perception (speech recognition in speech-shaped noise) and to quantify the degree of change in speech-in-noise recognition.

6.2. Methods

6.2.1. Participants

Thirteen normal hearing adults (21-30 years of age) without any evidence of hearing or ear disorder, corresponding to the ISO standard definition of otologically normal participated in this study. Acceptance as participants was based on otoscopy, pure-tone audiometry by air conduction (0.5, 1, 2, 3, 4, 6, 8 kHz) and bone conduction (0.5, 1, 2 kHz), tympanometry and acoustic reflex testing, and a simple screening questionnaire concerning medical and neuro-otological history. Additionally, their threshold of audibility to broad-band noise was also measured. The consent form was signed by each participant after the nature of experimental procedure was explained. This study was approved by the Institute of Sound and Vibration Research (ISVR) Human Experimentation Safety and Ethics Committee. The ear with better thresholds or right ear (if both ears have same thresholds) was tested for experimentation.

6.2.2. Acoustic reflexometry

Acoustic reflexometry was performed using a commercial immittance audiometer (GSI 33, version II). A tympanogram was plotted using a 226 Hz probe tone prior to reflexometry. Ipsilateral acoustic reflex thresholds (ART) for broad-band noise (BBN) were established by an ascending and descending 1-dB bracketing approach. ART was defined as the minimum SPL of BBN at which a reduction of not less than 0.03 ml in middle ear admittance can be measured at least 50% of the time.

6.2.3. Otoacoustic emissions

TEOAE were recorded using the Otodynamics ILO 292. The instrumentation is described in a previous chapter (Chapter 3, section 3.1.3). TEOAE were obtained in a

linear mode with stimuli consisting of clicks of 80 μs duration. The nominal stimulus level in the outer ear was set at 70 ±3 dB pe SPL with a click rate of 50/s and post-stimulus analysis in the range 2-20 ms. Responses to a total of 260 sets of clicks were averaged above the noise rejection level of 47 dB. A TEOAE was defined if its amplitude was 3 dB above the level of the noise floor, with overall reproducibility of 80% or more, and no bands less than 75% in four successive frequency bands ranging from 1 to 4 kHz.

TEOAE with contralateral acoustic stimulation was recorded as the broad-band noise (0.5-8 kHz) was presented at 30 dB SL to the opposite ear via an insert earphone (ER-3A). TEOAE measurements (with and without noise) were repeated twice and the order of measurement was random. TEOAE suppression (in dB SPL) was calculated by subtracting the overall amplitude of TEOAE in the presence of contralateral noise condition from that in the absence of contralateral noise. The spectrum of the click stimulus used to evoke TEOAE was inspected whenever the BBN was presented to the opposite ear during suppression measurements to check that there was no change in the click spectrum. It was also checked that the position of the probe did not alter during the recordings. The measurements were conducted in an acoustically treated double room (also used for speech intelligibility measurements). The overall TEOAE amplitude with contralateral acoustic stimulation was subtracted from that in the without contralateral acoustic stimulation condition to compute the suppression.

6.2.4. Speech intelligibility in noise

Speech intelligibility in noise was measured using Four Alternative Auditory Feature (FAAF) test (Foster and Haggard, 1987). This is a forced-choice word recognition task consisting of one list of 80 items in 20 sets of four alternatives. This is composed like rhyme tests on the binary feature principle (e.g., SUN, SUB, SUD, SOME or GET, BET, WET, YET or BAG, BACK, BAT, BAD). The target word occurs in the context of the carrier phrase, 'Can you hear (target) clearly?' The participant's task is to select the target word from the choice of four. The test was originally implemented in a pen and paper format but this has now been superseded with a touch-sensitive LCD panel to present the alternatives and gather responses.

The FAAF materials were replayed from a standard 16-bit computer sound card, at a sample rate of 20 kHz, routed via a Kamplex diagnostic audiometer to a TDH 50P earphone. They were presented against a background of steady noise that had been filtered to give a similar long-term spectrum to the keywords and delivered by the same earphone. The signal to noise ratio (SNR) in the test ear for each participant was altered adaptively during the test targeting a 70.7% correct score by means of a two-up-one-down algorithm (Levitt, 1971). The speech presentation level was fixed at 60 dB SPL while the ipsilateral noise level was varied adaptively in 2 dB steps. The 70.7% score was estimated from the mean of the final 8 reversals in the adaptive procedure. SNR score was defined as the difference in decibels between the SPL of the words and the SPL of the noise. SNR scores were measured (i) in the absence of contralateral noise, and (ii) in the presence of contralateral noise. It is important to note that there was always ipsilateral noise in the test ear during speech testing. To state it simply, SNR measurements were performed in monaural and binaural noise conditions. The order of SNR measurements was counterbalanced, and included a time interval of 15 minutes or more between two measurements. The contralateral broad-band noise was presented at 30 dB SL from the same audiometer via an insert earphone (ER-3A). The ipsilateral and contralateral noises were uncorrelated to eliminate the possibility of binaural unmasking.

6.2.5. Experimental protocol

Each participant was accepted after otoscopy, pure tone audiometry and tympanometry. The thresholds for BBN and ART were measured. TEOAE measurements without noise and with contralateral noise were conducted twice but in a random order. Similarly, the speech-in-noise measurements were conducted without noise and with contralateral noise in a random order (there was always noise in the ipsilateral/test ear). The order of TEOAE and speech in noise measurements was counterbalanced. The total test duration was approximately 1 hour.

6.2.6. Statistical analysis

The data were inspected before analysis to confirm if it is appropriate to use parametric statistics. TEOAE amplitudes and measured SNR scores showed normal distributions as computed using the Kolmogorov- Smirnov test. The TEOAE data collected from two recordings were compared to verify if they significantly different. Statistical analysis of

the data consisted of a paired samples *t*-test to determine the effects of contralateral noise on SNR score, and a Pearson's product moment correlation analysis to examine the relationship between the contralateral suppression of TEOAE and change in SNR score due to addition of contralateral noise.

6.3. Results

6.3.1. Acoustic reflex thresholds

The mean ART for BBN was 75 dB SPL (SD= 9.04). The mean level of contralateral noise presented for TEOAE and speech measurements was 54.2 dB SPL (SD= 4.68). These values confirm that the level of contralateral noise in both TEOAE and speech in noise measurements was well below the ART. An examination of individual data set also confirmed the same.

6.3.2. TEOAE suppression

The mean contralateral suppression value of overall TEOAE is presented in Figure 6.1. The mean suppressive effect was 1.73 dB (SD= 0.9). The SD on replication[8] for suppression measurements was 0.34 dB, showing repeatability of the measurements. The two suppression measurements were not significantly different ($p \geq 0.05$). It can be observed from the Figure 6.1 that the lower 95% confidence limit was above zero, indicating significant suppression.

[8] The difference in SD between two suppression measurements were divided by $\sqrt{2}$

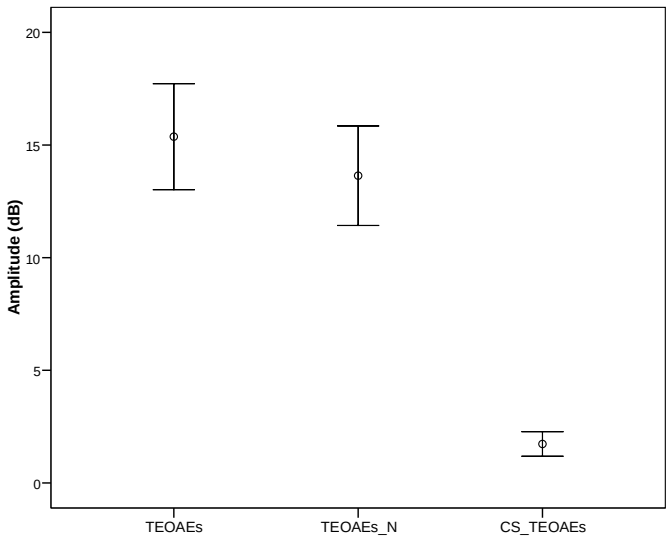

Figure 6.1. Mean and 95% confidence interval of amplitudes of TEOAE without and with contralateral noise (TEOAE_N) and suppression (CS_TEOAE).

6.3.3. Speech recognition in noise

The mean SNR scores without and with contralateral acoustic stimulation are plotted in Figure 6.2. It shows that in the presence of contralateral acoustic stimulation listeners can tolerate less favourable SNR to achieve the target score (70.7%). A paired *t*-test revealed statistically significant differences in SNR scores with and without contralateral acoustic stimulation ($t=7.53$; $p<0.01$). The advantage in SNR score was calculated as the difference in dB between SNR without and with contralateral noise. The mean difference in SNR scores due to addition of contralateral noise was 2.44 dB (SD= 1.17).

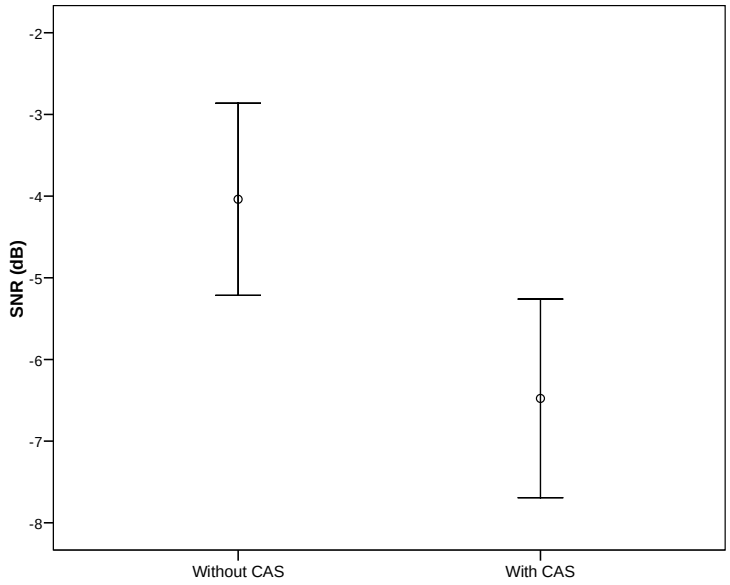

Figure 6.2. Mean and 95% confidence intervals for SNR scores without and with contralateral acoustic stimulation. The more negative the numerical value the better is the SNR score.

6.3.4. Relationship between TEOAE suppression and speech recognition in noise

Pearson's product moment correlation analysis showed a statistically significant relationship between the magnitude of TEOAE suppression and change (advantage) in SNR score due to contralateral acoustic stimulation ($r = 0.631$; $p<0.01$). The corresponding scatter diagram is shown in Figure 6.3, with the regression line superimposed through the data. An inspection of scatter plot indicates that the calculated correlation coefficient is due to contributions from all individual data and not from a few. It appears the individuals with greater TEOAE suppression require less advantageous SNR to achieve the target speech in noise score.

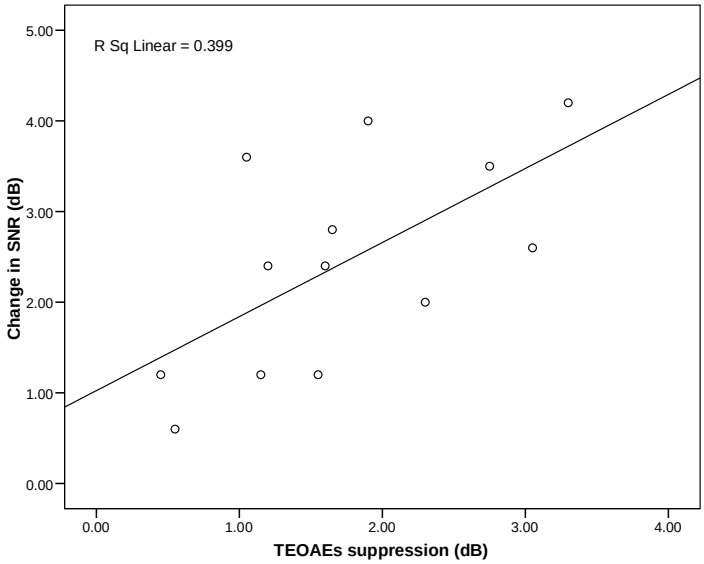

Figure 6.3. Change in SNR as a function of the magnitude of TEOAE suppression.

6.4. Discussion

6.4.1. Middle ear muscle reflex and other effects in CAS measurements

This discussion is similar to that in Chapter 5 (section 5.4.2). It is important to distinguish efferent effects of MOCB from middle ear muscle reflexes (MEMR) in experiments using contralateral acoustic stimulation to evoke MOCB reflex. In the present study, the mean difference between level of ART and level of contralateral noise was 20.8 dB (range 20-30). This large difference indicates that the level of contralateral acoustic stimulation is probably insufficient to evoke even weak MEMR, but the possibility of very weak MEMR cannot be ruled out as they may not be detected by clinical immittance meters. However, in general MEMR act to prevent intense acoustic stimulus from reaching cochlea principally at the low frequencies, in contrast, suppression usually occurs at frequencies above 1 kHz. In the present study the possibility of the MEMR during the contralateral acoustic stimulation measurements is remote, even if it cannot be entirely ruled out. In contrast, previous studies (Giraud *et al*., 1997; Kumar and Vanaja, 2004) on similar lines have attempted to exclude the possibility of MEMR in normal hearing listeners by theoretical arguments rather than actually measuring and comparing the levels of ART and contralateral sound in each participant.

Some of the other effects that could potentially cloud MOCB effects in contralateral acoustic stimulation measurements are masking due to interaural crossover and binaural unmasking. Interaural crossover due to contralateral sound can be eliminated as the participants had normal hearing thresholds; the insert earphone that was used to deliver the BBN has typical interaural attenuation value of 70 dB, and the presentation level of contralateral sound was rather low, 30 dB SL. The simultaneous presentation of BBN and speech-shaped noise during speech measurements may produce binaural unmasking (Gelfand, 1990, pp. 441-449), meaning that the detection of a signal in noise is improved when either the phase or level differences of the signal at the two ears are not the same as the masker. A possibility of binaural unmasking appears unlikely, as the ipsilateral and contralateral noises were uncorrelated.

6.4.2. TEOAE suppression

As expected, contralateral acoustic stimulation resulted in a reduction of TEOAE amplitude. The magnitude of the TEOAE suppression essentially provides an index of the power of MOCB feedback. The suppression effect seen provides additional evidence that, in the presence of contralateral noise, MOCB activation has an inhibitory effect, modulating outer hair cell mechanisms, interfering with the generation of otoacoustic emissions and promoting TEOAE attenuation. The suppression values found in the literature vary according to the intensity of the contralateral noise applied in the population studied (Collet *et al*., 1990, 1992; Hood *et al*., 1996).

6.4.3. Effect of contralateral acoustic stimulation on SNR scores

The SNR measurements were performed in the absence and presence of low level contralateral acoustic stimulation which is known to stimulate MOCB in listeners with normal auditory function. Participants performed better (needed less advantageous SNR) with contralateral acoustic stimulation to achieve the target correct score. On average, the improvement in SNR score due to contralateral acoustic stimulation was 2.44 dB. This shows that the effects of ipsilateral background noise on the speech recognition scores can be limited by addition of contralateral noise which stimulates the MOCB. This anti-masking effect of the MOCB corresponds to an improvement of about 11-15% in speech recognition scores at typical conversation levels, according to FAAF test normative data (Foster and Haggard, 1987).

The observed improvement may be compared with previous studies that reported similar benefits in speech intelligibility (Giraud *et al*., 1997a; Kumar and Vanaja, 2004; Zeng *et al*., 2000) due to methodological differences. Compared to previous studies, the present study used a more contemporary method and challenging situation (speech-shaped noise) to measure the speech perception in noise and used an LCD panel, a better mode (than the verbal method) to record the participant responses. Giraud *et al*. (1997b) reported an improvement of 5-10% in speech intelligibility scores with contralateral acoustic stimulation in normal hearing listeners. The speech measurement materials and methods were different than the present study. Importantly, they did not report the vocal threshold level in quiet that gave a 100% correct score, 10 dB above which the speech in noise measurements were performed. Without this information the magnitude of benefit in speech perception in noise in their study cannot be compared with the present findings. Zeng *et al*. (2000) reported poor speech in noise recognition in the operated-ear compared to non-operated ear in three out of four vestibular neurectomy patients tested, but this finding was confounded by the hearing loss. Kumar and Vanaja (2004) measured speech identification scores of English monosyllabic words in non-native English speaking children aged 10-12 years. Harkrider and Smith (2005) reported that the individual differences in speech in noise recognition cannot be accounted by the auditory efferent activity. This could be primarily due to the fact that the acceptable noise level (ANL) test characterizes the maximum level of background noise an individual is willing to accept while listening to running speech without becoming tense or tired. The relation between ANL and other direct speech-in-noise tasks has not been investigated. In the light of present findings and related evidence from previous reports (in spite of methodological differences and confounds) it can be asserted that stimulation of MOCB via contralateral sound helps in reducing detrimental effects of background noise on speech perception and thus aids in better understanding of speech in noisy backgrounds. However, it is likely that the method of speech measurements may have some effects in quantifying the anti-masking benefits of MOCB.

6.4.4. Relation between of MOCB feedback and speech recognition in noise

Improvements in SNR scores due to contralateral acoustic stimulation correlated with the contralateral suppression of TEOAE. This correlation indicates that the participants

with the most effective MOCB feedback were those in whom the speech perception in noise improvements (requiring less advantageous SNR to achieve the target score) with the contralateral acoustic stimulation was the strongest. Nevertheless, correlation based methods are not a very strong way of testing a hypothesis. A statistically significant correlation between the suppression of TEOAE and speech perception in noise does not necessarily mean an underlying fundamental linkage between them. This is certainly one of the limitations of the phenomenological approach that is encountered when physiological bases of perception in humans are investigated non-invasively. Consequently, it is necessary to critically analyse to what extent these results corroborate with various electrophysiological and related psychoacoustic experiments in humans and animals in order to establish an accurate interpretation of the observed relation between MOCB activity and speech recognition in noise.

Although the appropriate neurophysiologic bases of speech perception in noise remain to be established, the contribution of MOCB may be derived from a large body of physiologic experiments. For example, MOCB feedback reduces the response of the auditory nerve to the background sound and thereby reduces neural adaptation of the afferent fibres. This in turn allows a greater response to a transient sound. Efferent activity can produce substantial increase in discriminability of transient signals in noise (Kawase *et al*., 1993; Winslow and Sachs, 1987). May and MacQuone (1995) proposed that the neural representation of complex sounds, like speech, is based on a combination of rate responses that encode the level of pure tone stimuli across populations of auditory fibres, and efferent feedback may preserve the rate representation of complex sounds in noise by reducing neural sensitivity. The present results are consistent with these propositions: the response of afferent fibres to speech signals, which are composed of transients, improve with the presence of a binaural noise. Second, MOCB fibres could play a role in the spectral and temporal analysis and intensity coding of acoustic signals. Even though there is no direct evidence of the contribution of MOCB in temporal gap detection in normal listeners or vestibular neurectomy patients, OAE suppression studies have demonstrated that, in addition to reduction in amplitude, a contralateral sound induces a shift in phase and decreases TEOAE latency, presumably by shortening OAE generation time (Giraud *et al*., 1996). This suggests that MOCB feedback increases cochlear temporal resolution and thereby, improves the ability to interpret rapid amplitude fluctuations, such as those embedded in speech signal with fluctuating

background noise. Benefit of MOCB input in listeners with normal auditory function and lack of this benefit in human listeners with de-efferented system, such as, vestibular neurectomy has been reported in tone detection in noise, intensity discrimination in noise and other related psychoacoustic tasks (Micheyl *et al*., 1997; Micheyl and Collet, 1996; Zeng *et al*., 2000). Collectively, the observed correlation between TEOAE suppression and the corresponding improvement in speech recognition in noise appear to confirm the hypothesized relationship between the MOCB feedback and speech perception in noise.

6.5. Summary and Conclusions

TEOAE and speech in noise measurements were performed with and without contralateral acoustic stimulation. Contralateral acoustic stimulation significantly reduced the amplitudes of TEOAE and improved speech recognition in noise (i.e., required less SNR to achieve the same intelligibility). The present findings tend to confirm the hypothesis that MOCB feedback helps in reducing detrimental effects of background noise on speech recognition, hence suggesting an anti-masking role of MOCB in speech recognition in noise in humans with normal auditory function. This anti-masking function of MOCB in normal listeners can be quantified using behavioural speech in noise measurements in addition to TEOAE suppression measurements.

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1. Overview

This chapter summarizes the thesis work and presents a simple model for DPOAE mechanisms with reference to non-stimulus related variables acting on the cochlea. It also proposes a conceptual framework to enhance the power of DPOAE-based measurements of cochlear and efferent functions. Another indirect, but important fundamental implication of the present work to locate the source of fine structure in DPOAE, is also briefly addressed.

The primary goal of the thesis was to examine the conventional and mechanism-based OAE measures, which are expected to be particularly sensitive to small changes in cochlear physiology and efferent system functioning in human subjects. DPOAE reflect cochlear non-linear response properties and are sensitive to cochlear damage in a frequency specific manner. It is now accepted that DPOAE are composed of two different components: wave- and place-fixed emissions representing distortion and reflection sources respectively. To date, the significance of this scientific knowledge has not been explored in clinical studies or for diagnostic purposes. Also, this knowledge has been sparsely applied to laboratory experimental work. This translational research aimed at applying current knowledge of wave- and place-fixed components to answer some basic questions with regard to the evaluation of the cochlea and efferent auditory system. Additionally, this project incorporated the utilization of conventional OAE measures to assess cochlear and efferent auditory system functioning.

Attempts were made to change cochlear functioning in two non-invasive ways: (i) radiation from mobile phone and (ii) body position. While body position is a well known factor to induce changes in cochlear functioning, the potential effect of mobile phone radiation on cochlear functioning is not well known. Therefore, the effect of mobile phone radiation constitutes an important question in its own right. Efferent system functioning was measured by conventional TEOAE suppression and via novel DPOAE techniques. The functional relevance of the efferent auditory system in speech perception

in noise was also examined, in order to address the clinical significance of measuring OAE suppression.

The experiment (in Chapter 1) used both DPOAE and TEOAE to study the potential effects of EMF exposure on the auditory function. 2F1-F2 DPOAE is mixture of both wave- and place-fixed components, while TEOAE is primarily composed of place-fixed emissions (Shera and Guinan, 1999). The second and third experiment on posture-induced changes in cochlear mechanisms and efferent effects respectively used DPOAE but separated its components to define the changes more accurately. The results suggest that the place-fixed components show more changes than the wave-fixed components in relation to subtle changes in cochlear mechanisms (within the context of Chapter 4 and 5). Therefore, the final experiment (in Chapter 6) for assessing the role of efferent auditory system in speech perception used TEOAE to measure the efferent effects.

The following sections present key findings and conclusions from each experiment of the thesis.

1. *Measurement of changes in auditory functions due to EMF exposure.*

   Potential changes in auditory function (particularly cochlear functioning) due to EMF exposure from UMTS phones were evaluated by a within-subject study in a double-blind design. The test battery aimed at sensitive and comprehensive evaluation of the auditory system. This experiment was conducted within the consortium of EMFnEAR framework while at the same time aiming to track down the potential changes in cochlear functioning by conventional OAE measurement techniques. The administration of genuine and sham exposures was on separate days (at least 24 hours apart) and was counterbalanced in order, with the test participant and tester both blind to the condition being used. Importantly, the statistical analysis of the data was also performed blind. Results suggest that there was no significant effect of phone exposure on any of the measures, except hearing thresholds at high frequencies (6 and 8 kHz). However no other measures showed any sign of effect, hence no corroboration was found for the audiometric result. Although the presence of possible effects on hearing thresholds cannot be dismissed entirely, the current evidence is not sufficiently strong to conclude that there are adverse effects on hearing thresholds.

Nevertheless, the hypothesis that exposure to UMTS mobile phone radiation may induce potential changes in auditory functioning cannot be completely rejected.

Despite the difference in EMF exposure techniques compared to the present experiment Parazzini *et al.* (2005b) found no change in wave- and place-fixed components following exposure to GSM phones. Relating this null finding to the present finding of no change in conventional OAE based measures, it may be inferred that if wave- and place-fixed emissions do not show any cochlear dysfunction, conventional OAE measures may also not reflect any changes in cochlear functioning. In other words, changes in cochlear functioning would be first revealed by measurement of wave- and place-fixed emissions rather than conventional OAE tests.

2. *Measurement of posture-induced changes in cochlear functioning.*

   As expected, DPOAE amplitudes changed with the manipulation of body position due to change in ICP. However, present findings highlight the change in DPOAE phase gradient and differential change in the wave-and place-fixed components due to change in body position. Specifically, place-fixed components are more vulnerable due to change in body position than wave-fixed components. DPOAE phase gradient appears to increase by changing the body position from sitting to head-down, implying that the wave-fixed component becomes more dominant. ICP-induced cochlear changes predominantly affect the contribution of place-fixed components in 2F1–F2 DPOAE. Consequently, measurement of DPOAE components reduces the variability in measurement of ICP induced cochlear changes. The hypothesis that posture-induced changes in cochlear function have differential effect on wave- and place-fixed components DPOAE is accepted.

3. *Measurement of efferent induced changes in wave- and place-fixed components.*

   DPOAE are composed of two components that arise from two fundamentally different mechanisms; consequently, CAS may plausibly alter the two components differently. This experiment examined the effect of CAS on DPOAE and it provides evidence that CAS differentially affects wave- and place-fixed components without changing the dominance of the wave-fixed

components in 2F1–F2 DPOAE. Compared to wave-fixed components, the place-fixed components are relatively more influenced by the MOC (activated by CAS). Measurement of suppression of DPOAE components compared to DPavg reduces intra- and inter-subject variability. The lack of CAS effect on the phase of composite DPOAE may mean that MOC activation by contralateral stimulation does not produce any significant change in the group delay of composite DPOAE. The present findings suggest that considering the separation of components has the potential to improve the sensitivity of DPOAE-based assay of efferent functioning and would offer insight into several interesting and unclear efferent mechanisms. The hypothesis that contralateral acoustic stimulation has differential effects on wave- and place-fixed components DPOAE is accepted.

4. *Measurement of contralateral suppression of TEOAE and its functional relevance.*

   TEOAE and speech in noise measurements were performed with and without contralateral acoustic stimulation. Contralateral acoustic stimulation significantly reduced the amplitudes of TEOAE and improved speech recognition in noise. The present findings tend to confirm the hypothesis that MOCB feedback helps in reducing detrimental effects of background noise on speech recognition even when the noise is speech-shaped, hence confirming an anti-masking role of the MOCB in speech perception in noise in humans with normal auditory function. This anti-masking function of MOCB in normal listeners can be quantified using TEOAE suppression measurements.

7.2. Proposed mechanism of generation of DPOAE when cochlear functioning is manipulated by non-stimulus related variables

In this section, the DPOAE generation model (proposed by Shera and Guinan, 1999) is extended to encompass the generation of DPOAE when there is a pathological agent that causes cochlear dysfunction (e.g., noise exposure or systemic disease) or non-pathological subjective factor that modulates cochlear functioning (e.g., body position or efferent suppression). Figure 7.1 illustrates the mechanism of generation of DPOAE when the cochlear mechanism is modified by pathologic or non-pathologic (non-stimulus) factors. The detailed mechanism of the generation of 2F1–F2 DPOAE in the

normal cochlea is described elsewhere (Shera and Guinan, 1999). Briefly, DPOAE is composed of distortion and reflection sources. Distortion (D), leading to wave-fixed components, arises near the overlap region of the F1 wave and peak of the F2 travelling wave. These waves then propagate forward to their characteristic frequency place, where they are slowed by the mechanics of the basilar membrane, causing the delay typical of reflection emissions. Some energy is presumed to be reflected back by the characteristic DP place (and any imperfections basal to it) via a reverse travelling wave to the base of the cochlea and emitted into the ear canal. These reflections (R) together constitute the place-fixed components. The wave- and place-fixed components combine to form the composite DPOAE in the ear canal. In the normal human cochlea, the amplitude of the wave-fixed components are generally greater than that of the place-fixed components (D>R) for 2F1–F2 DPOAE with frequency ratios around 1.2.

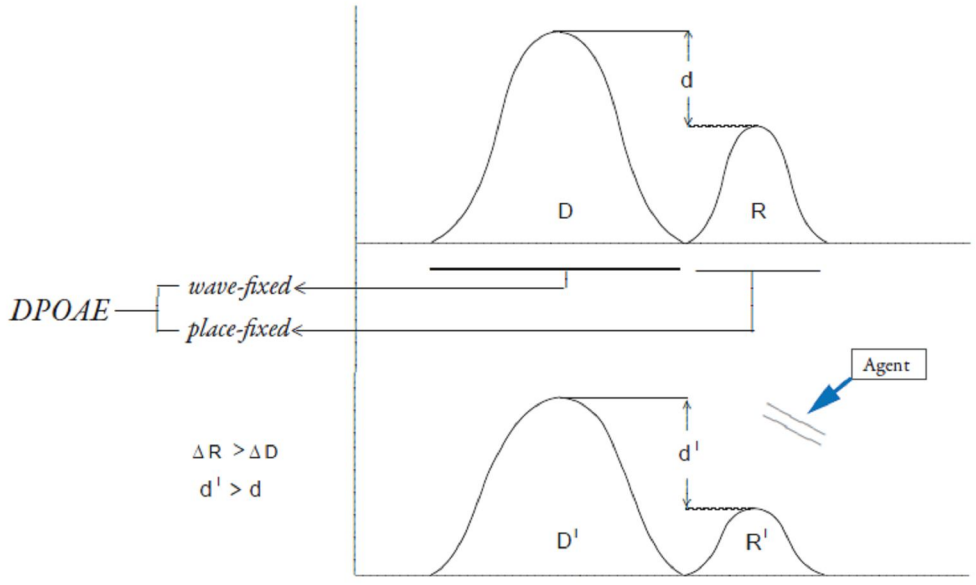

Figure 7.1. Schematic diagram of the generation of DPOAE when cochlear functioning is modified by a given pathologic or non-pathologic variable. The *x*- represents the amplitude in arbitrary units and *y*-axis is on nominal scale. The top panel shows the 2F1–F2 wave- and place-fixed components in normal cochleae, the bottom panel shows the predicted reduction in the 2F1–F2 wave- and place-fixed components due to an agent acting on the cochlea.

Because DPOAE represent cochlear non-linear distortion, in the event of an agent manipulating cochlear function, the agent would normally tend to affect cochlear non-linear properties when measured via DPAOE. As non-linearity in DPOAE generation is most evident in the overlap region the agent would cause reduction of distortion sources (D') and thereby wave-fixed components. Because part of the DP wave travels forward to form reflection sources, the general reduction of distortion sources would also lead directly to reduction of reflection sources. Reflection being a linear phenomenon, the already reduced energy in the DP travelling wave would be returned with equivalent energy by the reflection sites because in linear systems the output is proportional to the input. Depending upon the nature of the agent, for instance an agent causing cochlear insult or injury, the degree of reflections could be further impaired by cochlear abnormality at the reflection site. Cochlear abnormality (damage to OHC) could also smoothen the irregularities leading to reduction in reflection sources, thereby causing additional reductions in place-fixed components (R'). Overall, reflection components are expected to be reduced at least as much as, and probably more than, distortion components (see Fig. 7.1).

In summary, there is an inherent relationship between the wave- and place-fixed components in generating the 2F1–F2 DPOAE, in the sense that all components of the DPOAE originate from the distortion process and place-fixed components exist as a downstream by-product of the wave-fixed components. Therefore, ΔD must automatically lead to an element of ΔR that is at least as large as ΔD. The issue in question is whether the agent causes additional reduction of the place-fixed components so that ΔR is greater than ΔD. The efferent suppression and posture-induced cochlear change data support the notion that there is additional reduction of reflection sources and hence, place-fixed components ($\Delta R > \Delta D$). The greater reduction of the place-fixed components than wave-fixed components means that the wave- vs place-fixed difference (*d* in Fig. 7.1) would be larger when an agent interferes with cochlear function.

The key predictions from this simple model are:

1. The reduction in DPOAE amplitude (Δdp) is a combination of reduction in wave- (ΔD) and place-fixed (ΔR) components. This combination is dependent on phase of DPOAE. Mathematically, $\Delta dp = \Delta D + \Delta R$, where all terms are complex quantities.

The DP phase will, of course, tend towards the phase of the component with the greater amplitude. It is important to note that, because of the phase characteristics, the amount of change in the composite DPOAE amplitude may not necessarily be more than the change in individual components. The components may tend to cancel one another, if they are in phase opposition.

2. The reduction in place-fixed components (ΔR) would be usually at least as large as the reduction in wave-fixed components (ΔD) due to any non-stimulus related agent (e.g., noise exposure, posture-induced changes, etc.). In other words, agents that would affect distortion sources would essentially reduce reflection sources.
3. In 2F1–F2 DPOAE, the reduction of place-fixed components (ΔR) is determined by the change in reflection sources at the DP place (and any imperfections basal to it), as well as the change in the distortion sources. It is also presumably dependent on the characteristic of the agent acting on the cochlea, particularly if the agent induces some sort of anatomical malformations leading to change in the size, shape and/or spatial arrangement of cochlear microstructure (e.g. disruption of structure of stereocilia of OHC caused by noise damage).

The data from the efferent suppression and posture-induced changes in cochlear function experiments support the present model. For instance, in both the experiments, a greater reduction in place-fixed components compared to wave-fixed components is observed. This model might be applied to predict changes in the cochlea that may occur due to other non-stimulus related factors. For instance, ageing could cause general degeneration and degradation in cochlear microstructure and passive motion of the basilar membrane, thereby leading to greater reduction in place-fixed than wave-fixed components. The validity of this model across different pathologic and non-pathologic conditions remains an important open question. The systematic examination of this model is currently limited due to the lack of sufficient studies, particularly, the effect of pathophysiological changes on wave-and place-fixed components. Nevertheless, several studies on aspirin, quinine and other ototoxic drugs (Martin *et al*., 1988; McFadden and Pasanen, 1994; Parazzini *et al.*, 2005a; Wier, Pasanen, and McFadden, 1988) have found that SOAE and SFOAE disappear quite early while composite DPOAE could remain unchanged. This effect can be explained by predictions from the present model, assuming place-fixed (reflection) components are responsible for the generation of SOAE and SFOAE. Some of the changes in OAE due to manipulation of ICP (by

changing body position) might have stemmed from changes in the middle ear pressure. However, it is unknown why this would cause differential changes in the components. ICP can induce changes in cochlear mechanisms is known from patients with endolymphatic hydrops (Cianfrone *et al*., 2000). Endolymphatic hydrops is a disorder of the vestibular system of the inner ear. It results from abnormal fluctuations in the fluid called endolymph, which fills the hearing and balance structures of the inner ear. These patients in initial stages show signs of fluctuating low-frequency hearing loss. It would be interesting to investigate if these patients show reduced place-fixed components relative to wave-fixed components. Finally, it would be interesting to see how this model stands when cochlea is genetically modified.

Because place-fixed components are (arguably) responsible for the generation of at least part of the fine structure in DPOAE, indirectly this model would predict the abolition or reduction of fine structure due to cochlear pathology. This can be at least partially supported by studies that have indicated the disappearance of fine structure due to cochlear damage (Mauermann *et al*., 1999a,b; Talmadge *et al*., 2000).

7.3. Framework to enhance the utility of DPOAE-based measures

The clinical interpretation of the DPOAE is not usually based on a comprehensive understanding of their origin. The understanding of two different mechanisms and the primary origin of the DPOAE should be the foundation of clinical interpretation of the 2F1–F2 DPOAE, in terms of frequency selectivity and site of lesion. The aim of accurate clinical interpretation of DPOAE makes measurement of components of 2F1–F2 DPOAE important.

This section suggests the clinical significance of similarities and differences between wave-and place-fixed components. Although DPOAE is composed of wave- and place-fixed components, the clinical utility of DPOAE has traditionally focused on the measurement of the amplitude of composite DPOAE. Current knowledge suggests that DPOAE is composed of two different components that not only arise from two different cochlear locations but also from two different mechanisms. Consequently, they would have different dependency on the nature and site of any pathology. Wave-fixed components depend on the intrinsic nonlinear characteristics of OHC, especially, the

cilliary bundle displacement versus hair cell voltage transduction function (Shera, 2004). In contrast, place-fixed components indirectly depend on cochlear non-linearity but more directly depend on the size, shape and spatial arrangement of anatomical microstructure near the peak of the secondary DP travelling wave and the coherent reflection mechanism; hence, they may be relatively more sensitive to the gain of the cochlear amplifier. Therefore, wave-and place-fixed components potentially manifest different dependency on cochlear pathologies.

The following key similarities and differences between wave-and place-fixed components form the basis for potentially improving the utility of DPOAE-based measurements.

1. The DPOAE components share a common dependency on the reverse pathway from cochlea to the ear canal and are sensitive to changes in the cochlea, middle ear and ear canal.
2. Both components in varying degrees depend on cochlear non-linear properties and also share a general first order dependence on the cochlear amplifier.
3. As highlighted several times, they arise from different cochlear locations and different mechanisms.

For hearing screening purposes, the differences between wave- and place-fixed components may not be very relevant as both components share a common first order dependence on the cochlear amplifier. In contrast, separation of components is more important for determining frequency specificity of cochlear pathology as frequency specificity in composite DPOAE is compromised by the spatial blurring of two components. Due to their sensitivity to cochlear amplification, place-fixed components would presumably be relatively more important for monitoring of changes in cochlear functioning over time. Thus, separation of components may help clinical interpretation of DPOAE in terms of frequency specificity and site of lesion.

The ability of any test to define the degree of impairment depends on the response repeatability within and across subject. Because wave- and place-fixed components mix depending upon their relative phase to produce the composite DPOAE measured in the ear canal, separating these components may reduce intra-subject variability. Because clinical measurements are usually expressed at audiometric frequencies, reduction in intra-subject variability would tend to increase the inter-subject repeatability. In fact, the

enhanced repeatability with component separation is also important in laboratory experiments. For instance, in this thesis, both posture-induced and efferent-induced cochlear changes experiments showed increased repeatability with component separation. Additionally, component separation eliminates suppression enhancements in efferent suppression measurements, because it resolves phase cancellation issues. However, this was not true for at least one participant.

7.4. Source of fine structure in DPOAE

Although beyond the present scope, this work provides some indirect evidence with regard to the source of DPOAE fine structure. Several studies (Gaskill and Brown, 1996; Heitmann *et al*., 1998; Mauermann *et al*., 1999a, 1999b; Stover, Neely and Gorga, 1999; Talmadge, Tubis, and Long 1998; Kalluri and Shera, 2001) suggested that the 2F1–F2 DPOAE fine structure found in humans is the result of the interference of the two-generation sources at the F2 and Fdp sites (i.e., interference hypothesis). However, these studies could not rule out the possibility of an alternative hypothesis (i.e., place-fixed hypothesis); specifically, the fine structure of 2F1–F2 DPOAE is determined by the local impedance properties of the cochlear partition at the Fdp place. For instance, when the Fdp place is damaged the fine structure will disappear; conversely, when the F2 place is damaged with an intact Fdp place, the fine structure can be still observed as long as DPOAE can be recorded. The existence of fine structure in SFOAE supports this alternative hypothesis. Theoretically, if the DPOAE fine structure were generated only by the so called "constructive and destructive" interference of the two generation sources, fine structure of SFOAE would not be expected because it has only place-fixed components. In fact, fine structure of SFOAE can easily be demonstrated in human subjects (Harris and Brown, 1994; Stover and Norton, 1992).

Some of the present work (Chapter 4 and 5) indirectly supports the place-fixed hypothesis. The observation of clear fine structure in the 2F1–F2 place-fixed components (generated at the particular characteristic DP frequency place) along the cochlear partition, under a given test protocol cannot be explained by the interference hypothesis. This observation rather supports the idea that place-fixed components are responsible for DPOAE fine structure. Nevertheless, the interference of the two DPOAE components certainly can have influence on the fine structure, as long as 2F1–

F2 DPOAE and place-fixed components do not have identical fine structure patterns. In future work, the detailed statistical comparison of the fine structure characteristics between DPOAE and place-fixed components in normal and cochlear impaired population could shed some light into the locus of DPOAE fine structure. However, the co-existence of both interference and place-fixed mechanisms contributing to DPOAE fine structure seems to be best supported by evidence available to date.

7.5. Conclusion and future directions

For any OAE test to be maximally useful as an assay of cochlear and efferent function, it is imperative to understand OAE characteristics over the entire range of frequencies and intensities with reference to the generation mechanisms. Also, understanding how these measures are dependent upon different pathologies remains an important question. Admittedly, this thesis focuses on only one aspect of the problem for limited stimulus conditions; specifically understanding how separate estimates of DPOAE component measurements and conventional OAE measures differ in probing cochlear and efferent functions. Nevertheless, results in this thesis provide emerging experimental support for the use of DPOAE component measurements. Future research might consider the ways to improve the accuracy in quantifying phase gradient of the place-fixed components and the measurement of phase gradient of DPOAE components to study cochlear delay properties in hearing impaired subjects. Continued research towards understanding the mechanisms of emission generation will improve the power and specificity of OAE as non-invasive probes of cochlear and efferent function. Such research may encompass cochlear modelling to investigate potential generation mechanisms in mathematical detail, coupled with experimental work to test hypotheses derived from modelling. As indicated above, experimental work utilising genetically modified laboratory animals may help to unravel the complexities of cochlear mechanics leading to generation of the various forms of OAE. The successful translation of such knowledge into clinical practice could require development of a simple and quick OAE measurement system that would provide direct estimates of wave- and place-fixed components.

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APPENDIX 3.1

HEALTH QUESTIONNAIRE.

Participant Code:

Please circle the correct answer:

1. Do you think that your hearing is normal? Yes / No
2. Have you ever had any persistent problems with your ears or hearing, for example discharging ears or earache? Yes / No
3. Do you have tinnitus or ringing ears? Yes / No
4. Have you been exposed to loud noises, for example at work, gunfire or explosives? Yes / No
5. Do you attend loud night clubs frequently or have you been in past 48 hours? Yes / No
6. Are you suffering from or have you recently had a cold? Yes / No
7. Have you ever had attacks of dizziness or loss of balance related to vestibular disorder? Yes / No
8. Are you receiving any medical treatment or medication that may affect your hearing? Yes / No
9. Have you consumed alcohol or other drugs in the last 24 hours? Yes / No
10. Is there any history of hearing loss in your family? Yes / No

Please provide any other details:

APPENDIX 3.2

SUBJECT CONSENT FORM

Consent form to be completed by adult subjects taking part in an experiment

(Adults are 18 years of age or older.)

Exposure Number: .....

University of Southampton
Institute of Sound and Vibration Research

This consent form applies to a subject volunteering to undergo an experiment for research purposes. The form is to be completed before the experiment commences.

I, ...
of .....
(address or department)

consent to take part in the experiment on effects of mobile phone exposure on auditory system, by Mr Srikanta Mishra under the direction of Prof. Mark E Lutman at the ISVR Hearing & Balance Centre, Southampton.

The purpose and nature of this experiment have been explained to me. I understand that the investigation is to be carried out solely for the purposes of research. I am willing to act as a volunteer for that purpose on the understanding that I shall be entitled to withdraw this consent at any time, without giving any reasons for withdrawal. My replies to the above questions are correct to the best of my belief, and I understand that they will be treated by the experimenter as confidential.

Date: ..... Signed: .....
(Volunteer subject)

I confirm that I have explained to the subject the purpose and nature of the investigation which has been approved by the Human Experimentation Safety and Ethics Committee at the university.

Date: ..... Signed: .....
(Researcher in charge of experiment)

(Note: The data related to the experiments would be kept confidential. The confidentiality of all personal information which you provide during the course of the experiment will be ensured unless you consent to the disclosure of such information. It is further protected by the University's Data Protection Registrations.)

APPENDIX 3.3

HISTOGRAMS OF AVERAGED DATA

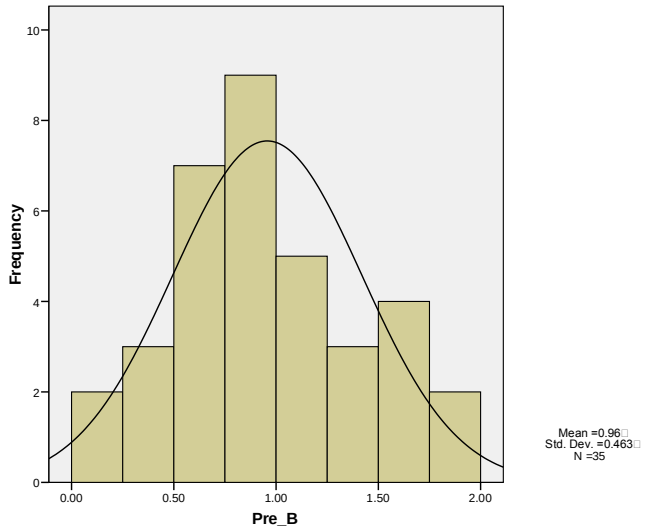

Fig. 3.1.1. Representative example of CAS effect of TEOAE histogram.

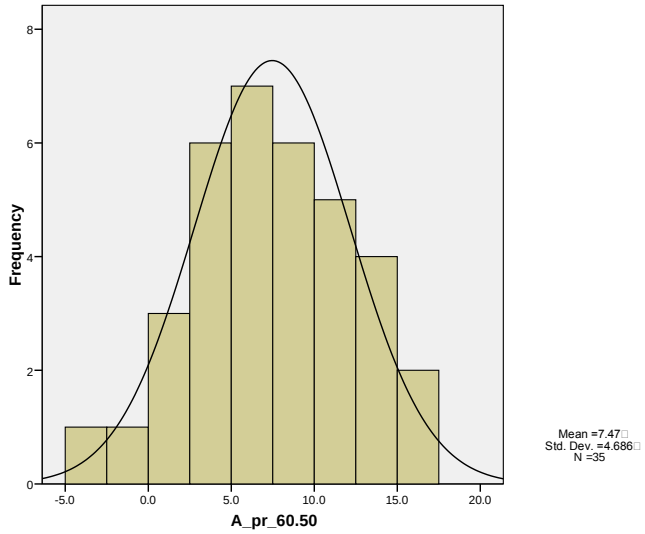

Fig. 3.1.2. Representative example of DPOAE histogram.

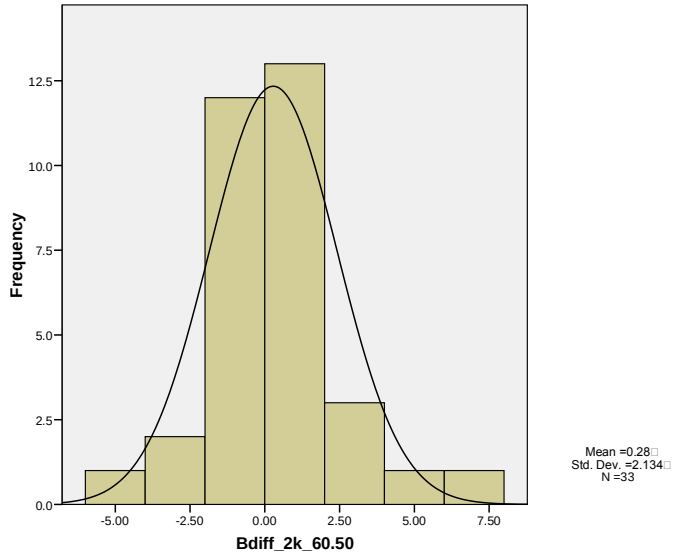

Fig.3.1.3. Representative example of DP growth histogram.

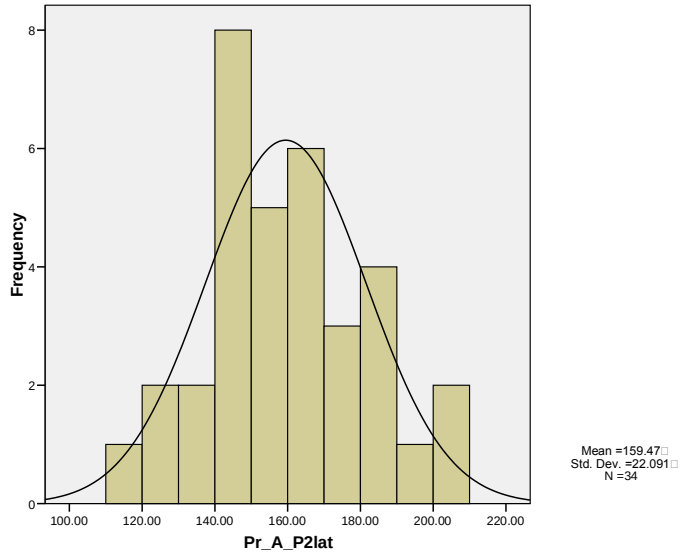

Fig.3.1.4. Representative example of DP growth histogram.

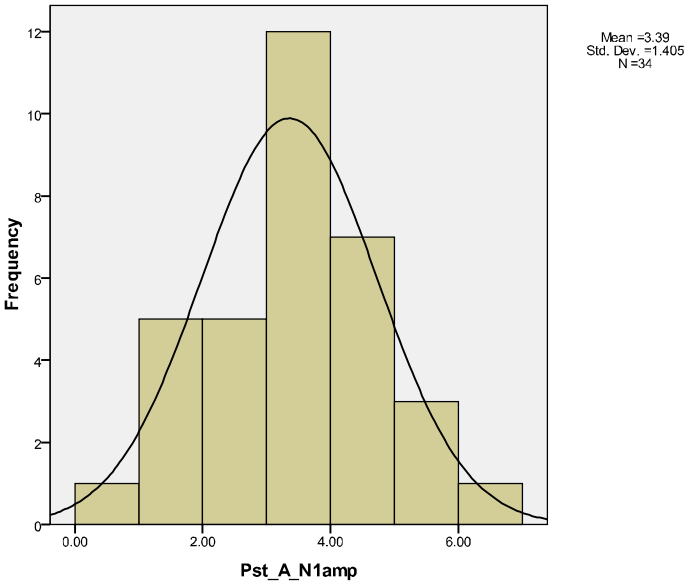

Fig.3.1.5. Representative example of ERP (N1 amplitude) histogram.

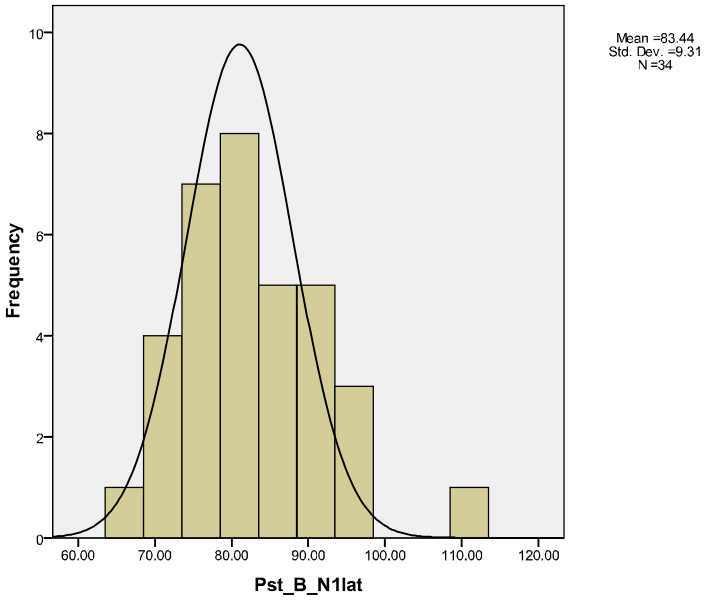

Fig.3.1.6. Representative example of ERP (N1 latency) histogram.

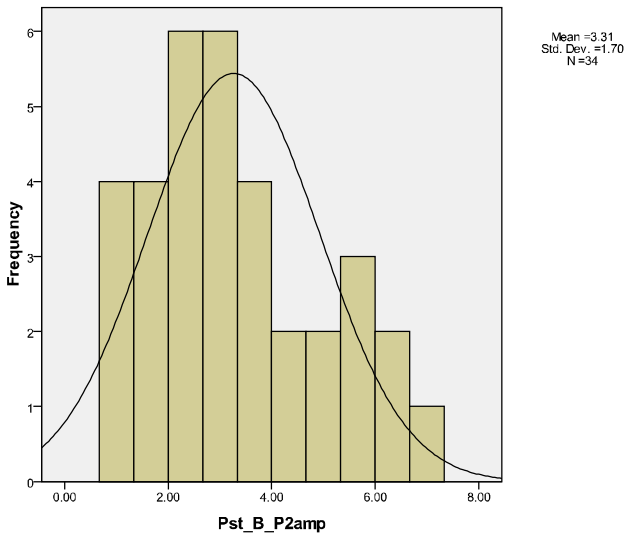

Fig.3.1.7. Representative example of ERP (P2 amplitude) histogram.

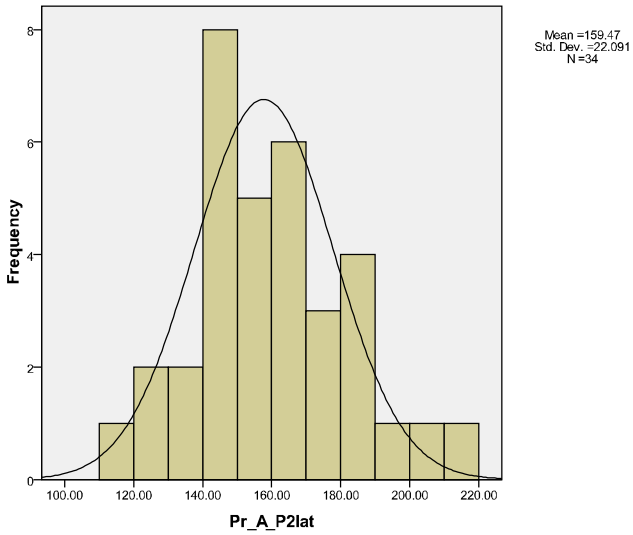

Fig.3.1.8. Representative example of ERP (P2 latency) histogram.

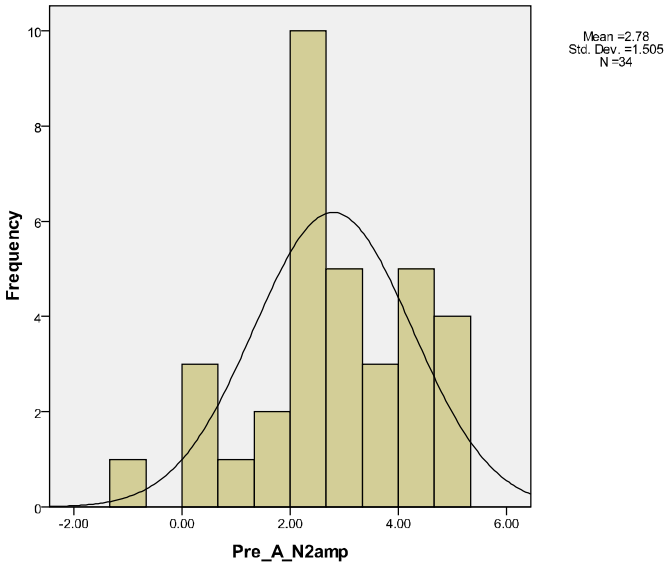

Fig.3.1.9. Representative example of ERP (N2 amplitude) histogram.

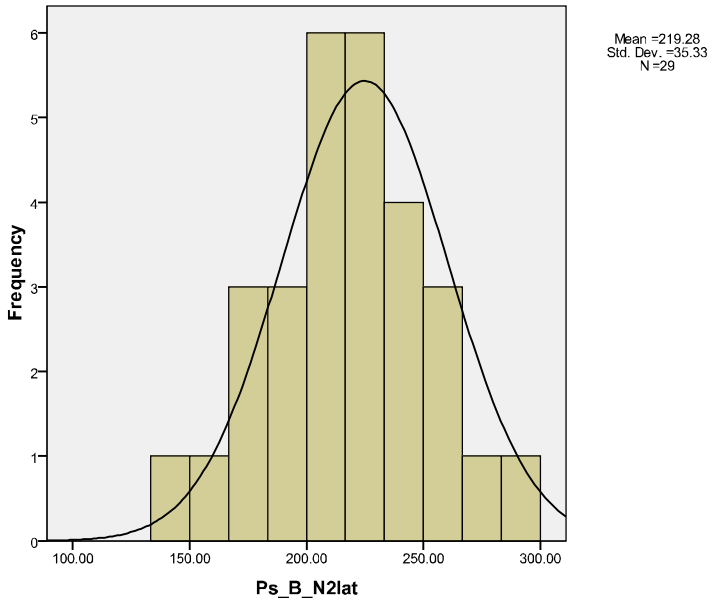

Fig.3.1.10. Representative example of ERP (N2 latency) histogram.

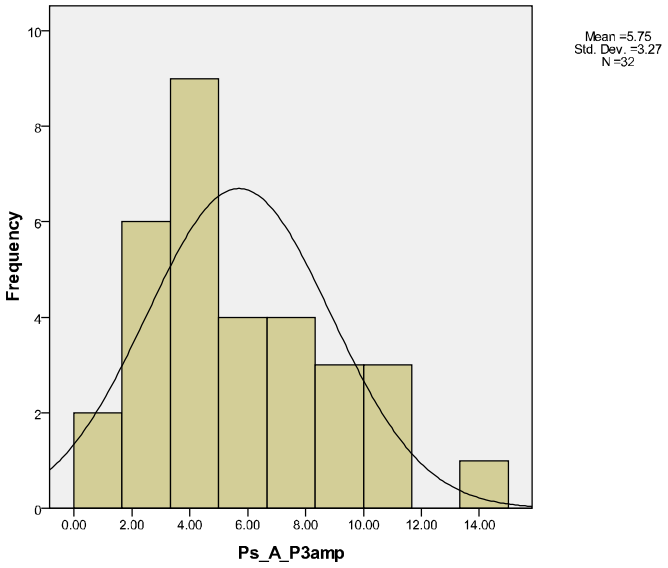

Fig.3.1.11. Representative example of ERP (P3 amplitude) histogram.

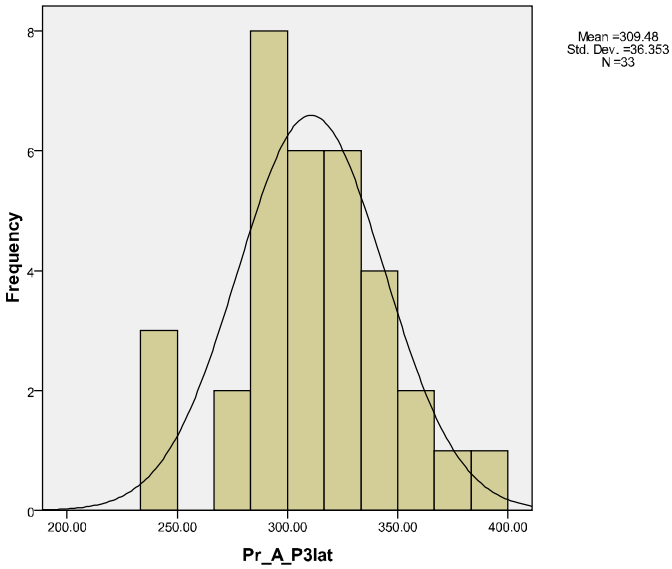

Fig.3.1.12. Representative example of ERP (P3 latency) histogram.

APPENDIX 4.1

MATLAB SCRIPTS FOR DPOAE DATA PROCESSING

Cleaning

```
%cd ('C:\Documents and Settings\user\Desktop')
clear all
cd ('C:\Separation program')

for i=1:1
   [filename, path]=uigetfile('*.dat;*', 'Pick a file');
   fid=fopen(filename,'r');
   for i=1:40
      line=fgetl(fid);
   end
   mat=fscanf(fid,'%32f',[20 inf]);
   mat=mat';
```

Program 1

```
cd ('C:\Documents and Settings\user\Desktop')
for i=1:1
   [filename, path]=uigetfile('*.dat;*', 'Pick a file');
   fid=fopen(filename,'r');
   for i=1:40
      line=fgetl(fid);
   end
   mat=fscanf(fid,'%32f',[20 inf]);
   mat=mat';
   f1=mat(:,1);
   f2=mat(:,4);
   Dp_amplitude=mat(:,8);
   Dp_phase=mat(:,10);
   Snr=mat(:,12);
   rad=Dp_phase*(pi/180);
   rad_unwrap=unwrap(rad);
   if strcmp ('dat',filename((length(filename)-2):length(filename)))
      filename = filename(1:length(filename)-4);
   end
   s=strcat(filename,' f1 f2 Dp_amplitude Dp_phase Snr radian_unwrap' );
   eval(['save ', s]);
   st=fclose(fid);
end
```

Program 2

```
function[Dp_complex_equation,Dp_distortion,Dp_distortion_no.
window,Dp_reflection,Dp_reflection_no.window,Dp_frequency_equation]=
unmixing(n,tcutoff,type,ratio,filename)

%function[Dp_complex_equation,Dp_distortion,Dp_distortion_no.
window,Dp_reflection,Dp_reflection_no. window,Dp_frequency_equation]=
 unmixing(n,tcutoff,type,ratio,filename)
%function of the unmixing algorithm according to Withnell et al Hear. Res. 178, 2003,
106-117
%input data: n:order of the recursive exponential filter
%           tcutoff: filter cutoff (recursive exponential filter)
%           type: if b means before, a after
%           ratio: f2/f1
%           filename: neme for saving the file
%all these vector are read from the data exported from the DPOAE recording system
and converter with loaddpfile %

cd ('c:\Documents and Settings\user\Desktop')
uiload;%chose one file obtained with loaddpfile
fs=32768;
%sample frequency of the system (hz)
deltaf=16;
%binwidth
N=fs/(deltaf);
%N number of points in frequency (2048 till fs no till Nyquist, no mirroring)
Max_frequency=deltaf*N;
%max frequency (32768 hz according to the article you have to go till fs, no till Nyquist)
f=[deltaf:deltaf:Max_frequency/2];
%frequency vector till Nyquist

%step 1: conversion of Amplitude and Phase in complex number; Amplitude in mPa
Phase %unwrapped in radians
Dp_amplitude_mPa=unitconv2(Dp_amplitude,'dBmPa');
Dp_phase_radian=Dp_phase*(pi/180);
Dp_phase_radian_unwrap=unwrap(Dp_phase_radian);       % unwrap function to avoid
jumps greater than pi
Dp_complex=complex(Dp_amplitude_mPa.*cos(Dp_phase_radian_unwrap),
Dp_amplitude_mPa.*sin(Dp_phase_radian_unwrap));

%step 2: linear interpolation of the data to obtain 16 hz of step between the Dp
frequency.
%With our way of recording we have a step of 16 Hz
Dp_frequency=2*f1-f2; random=rand(63,1);
Dp_frequency=(Dp_frequency+random);
Dp_frequency=sort(Dp_frequency);
Dp_frequency_equation=[Dp_frequency(1):deltaf:Dp_frequency(length(Dp_frequency))];
Dp_complex_equation=interp1(Dp_frequency,Dp_complex,Dp_frequency_equation,'lin
ear');
```

```
%step 3: the complex data is buffered with zeros from 0 to fs.
%No mirroring of the complex data is performed.
buffer_data=zeros(1,N);
index=fix(N*(Dp_frequency_equation./Max_frequency));
buffer_data(index)=Dp_complex_equation;

%step 4:moving average windowing. The data are windowed using a succession of 30
points wide (480 hz)
%Hanning windows. Successive windows were 15 data points apart.A IFFT was
performed on each windowed
%data set. The total IFFT is the sum of these individual IFFT.
%The time resolution is 30.5 micros. The time-domain waveform obtained from the
IFFT
%extended from 0 to 62.5 ms (2048 points multiplied by 30.5 micros).
Npoints=30;
shift=Npoints/2;
windowed_data=slid_hann(Npoints,shift,buffer_data);
windowed_timedata=ifft(windowed_data,N,2);
% IFFT of each windowed data
timedata=sum(windowed_timedata);  % analytic signal (total IFFT with moving average)
time_magn=abs(timedata);
  % envelope of the analityc signal (with moving average)
time_no. window=ifft(buffer_data,N);              % analytic signal (no moving average)
time_no. window_magn=abs(time_no. window);
% envelope of the analityc signal (no moving average)
t=[0:(1/Max_frequency):(N-1)*(1/Max_frequency)].*1000;  % time vector (in ms!)
figure;plot(t,time_magn,'r',t,time_no. window_magn);

%step 5: each IFFT is multiply by a n-order recursive exponential filter to remove
%components attribute to reflections within the cochlea (developed by Shera and Zweig
1993)
recursive_filter = recursive_exponential_filter(n,tcutoff,t);
matrix_recursive_filter=recursive_filter(ones(1,size(windowed_timedata,1)),:);
filtered_windowed_time=windowed_timedata.*matrix_recursive_filter;  %filter on each
windowed data
filter_time=time_no. window.*recursive_filter; %filter on time data no moving average

%step 6: An FFT is performed on each filtered IFFT.
%The FFT is performed on N value but only the first 0 to N/2 values are necessary (the
%values from N/2+1 to N-1 are redundant conjugates). The individual FFT are summed
to obtain
%the total FFT,i.e.the complex amplitude of the wave-fixed (or distortion) component.
filtered_frequencydata=fft(filtered_windowed_time,N,2);
Dp_distortion=sum(filtered_frequencydata); %complex amplitude of the wave-fixed
component (with moving average)
Dp_distortion_no. window=fft(filter_time,N); %complex amplitude of the wave-fixed
component (no moving average)
Dp_distortion_amplitude=unitconv2(abs(Dp_distortion(index)),'mPadB');
Dp_distortion_no.window_amplitude=unitconv2(abs(Dp_distortion_no.
window(index)),'mPadB');
Dp_equation_amplitude=unitconv2(abs(Dp_complex_equation),'mPadB');
```

```
%figure;plot(Dp_frequency_equation,Dp_distortion_amplitude,'r',Dp_frequency_equati
on,Dp_equation_amplitude); %plot of original Dp amplitude versus wave-fixed
component (with windowing)
%figure;plot(Dp_frequency_equation,Dp_distortion_no.
window_amplitude,'r',Dp_frequency_equation,Dp_equation_amplitude); %plot of
original Dp amplitude versus wave-fixed component (no. window)
%figure;plot(Dp_frequency_equation,unwrap(angle(Dp_distortion(index))),'r',Dp_freque
ncy_equation,unwrap(angle(Dp_complex_equation))); %plot of the original Dp phase
versus wave-fixed component (with windowing)
%figure;plot(Dp_frequency_equation,unwrap(angle(Dp_distortion_no.
window(index))),'r',Dp_frequency_equation,unwrap(angle(Dp_complex_equation)));
%plot of the original Dp phase versus wave-fixed components (no. window)

%step 7: the total place-fixed (or reflection) component is obtained by subtraction of the
%complex amplitude of the wave-fixed from the original data
Dp_reflection=Dp_complex_equation-Dp_distortion(index);
Dp_reflection_no. window=Dp_complex_equation-Dp_distortion_no. window(index);
Dp_reflection_amplitude=unitconv2(abs(Dp_reflection),'mPadB');
Dp_reflection_no.window_amplitude=unitconv2(abs(Dp_reflection_no.
window),'mPadB');

if type=='b'
   type='before';
elseif type=='a'
   type='after';
end
r=num2str(ratio);
str1=strcat('Dp Amplitude-Hanning',' (',type,'-',r,')');
str2=strcat('Dp Phase-Hanning',' (',type,'-',r,')');
figure;subplot(2,1,1);
plot(Dp_frequency_equation,Dp_equation_amplitude,'ro-
',Dp_frequency_equation,Dp_distortion_amplitude,'gs-
',Dp_frequency_equation,Dp_reflection_amplitude,'bd-
',Dp_frequency,Dp_amplitude,'k','LineWidth',1,'MarkerSize',2);
h=gca;set(h,'YLim',[-40 25]);title(str1);ylabel('dB SPL');
hold on;subplot(2,1,2);
plot(Dp_frequency_equation,unwrap(angle(Dp_complex_equation)),'ro-
',Dp_frequency_equation,unwrap(angle(Dp_distortion(index))),'gs-
',Dp_frequency_equation,unwrap(angle(Dp_reflection)),'bd-
',Dp_frequency,Dp_phase_radian_unwrap,'k','LineWidth',1,'MarkerSize',2);
h=gca;set(h,'YLim',[-40 10]);title(str2);ylabel('Radiaans');xlabel('2f1-f2 (Hz)');
legend('Dp','DpDistorion','DpReflection','Original',3);
%figure with hanning window

str1=strcat('Dp Amplitudelitude',' (',type,'-',r,')');
str2=strcat('Dp Phase',' (',type,'-',r,')');
figure;subplot(2,1,1);
plot(Dp_frequency_equation,Dp_equation_amplitude,'row',Dp_frequency_equation,Dp
_distortion_no.window_amplitude,,Dp_frequency_equation,Dp_reflection_no.window_
amplitude,'bd-',Dp_frequency,Dp_amplitude,'k','LineWidth',1,'MarkerSize',2);
h=gca;set(h,'YLim',[-40 25]);title(str1);ylabel('dB SPL');
```

```
hold on;subplot(2,1,2);
plot(Dp_frequency_equation,unwrap(angle(Dp_complex_equation)),'ro-
',Dp_frequency_equation,unwrap(angle(Dp_distortion_no.window(index))),'gs-
',Dp_frequency_equation,unwrap(angle(Dp_reflection_no.window)),'bd-
',Dp_frequency,Dp_phase_radian_unwrap,'k','LineWidth',1,'MarkerSize',2);
h=gca;set(h,'YLim',[-40 10]);title(str2);ylabel('Radians');xlabel('2f1-f2 (Hz)');
legend('Dp','DpDistorion','DpReflection','Original',3);
%figure without hanning window

Dp_distortion=Dp_distortion(index);
Dp_distortion_no. window=Dp_distortion_no. window(index);
% in this way all the vector exported have the same length.
 AmplitudeD=(Dp_distortion_no. window_amplitude)';
AmplitudeR=(Dp_reflection_no. window_amplitude)';
PhaseD=(unwrap(angle(Dp_distortion_no. window)))';
PhaseR=(unwrap(angle(Dp_reflection_no. window)))';
s=strcat(filename,'DpDR');
r= strcat(s,' Dp_frequency_equation AmplitudeD PhaseD AmplitudeR PhaseR');
eval(['save ', r]);


AmplitudeDw=(Dp_distortion_amplitude)';
AmplitudeRw=(Dp_reflection_amplitude)';
PhaseDw=(unwrap(angle(Dp_distortion)))';
PhaseRw=(unwrap(angle(Dp_reflection)))';
s=strcat(filename,'DpDRw');
r= strcat(s,' Dp_frequency_equation AmplitudeDw PhaseDw AmplitudeRw PhaseRw');
eval(['save ', r]);
```

Program 3

```
cd ('c:\Documents and Settings\user\Desktop')
uiload;
steponeD=AmplitudeD/10;
steptwoD=10.^(steponeD);
stepthreeD=mean(steptwoD);
averageD=10*(log10(stepthreeD))
steponeR=AmplitudeR/10;
steptwoR=10.^(steponeR);
stepthreeR=mean(steptwoR);
averageR=10*(log10(stepthreeR))
```

APPENDIX 4.2

HISTOGRAMS OF AVERAGED DATA

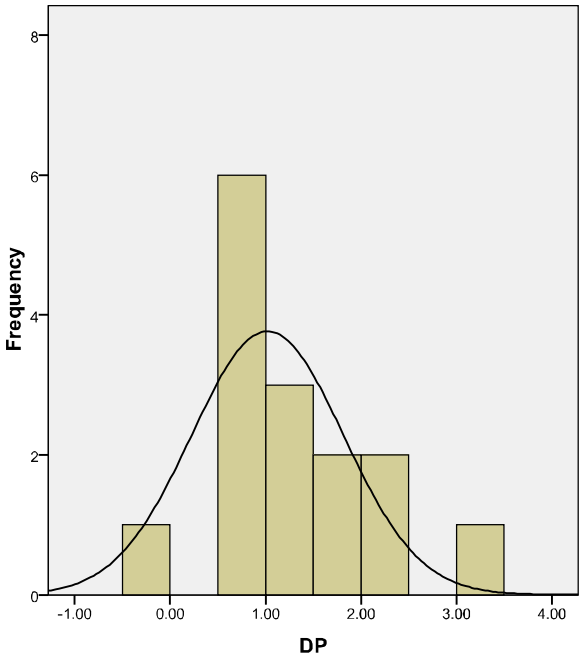

Fig. 4.1.1. Example of DPAOE histogram.

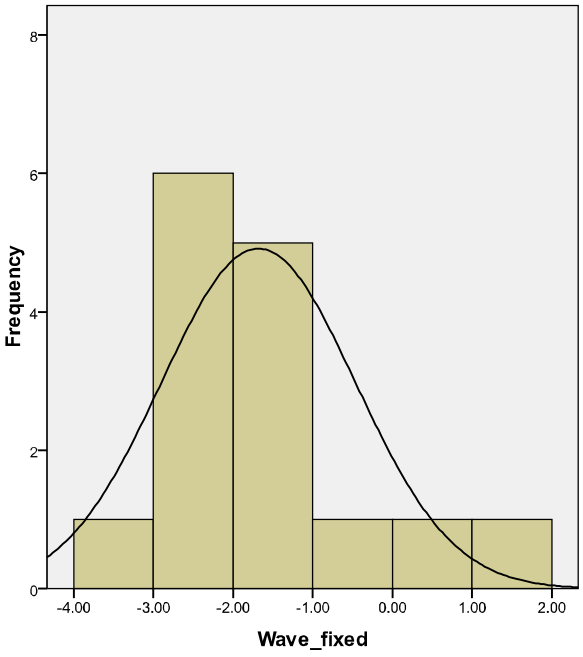

Fig. 4.1.2. Example of wave-fixed component histogram.

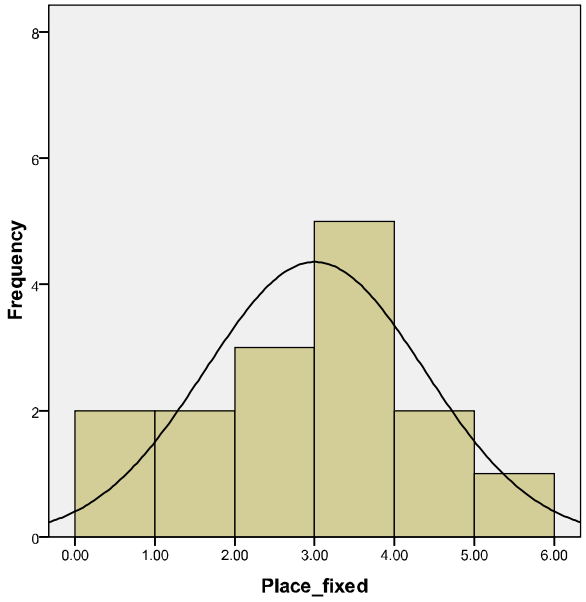

Fig. 4.1.3. Example of place-fixed component histogram.

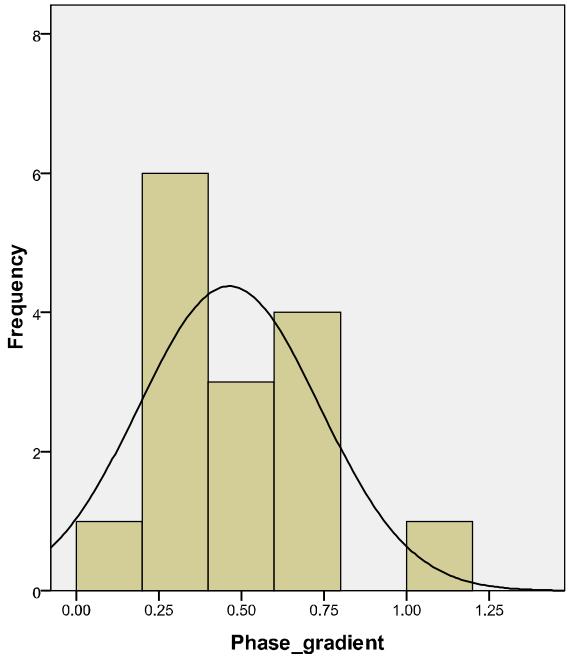

Fig. 4.1.4. Example of phase gradient histogram.

APPENDIX 5.1

HISTOGRAMS OF AVERAGED DATA

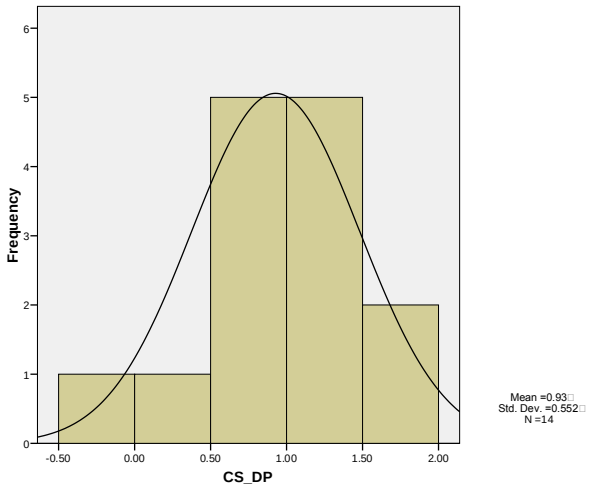

Fig. 5.2.1. Representative example of histogram of CS_DP data.

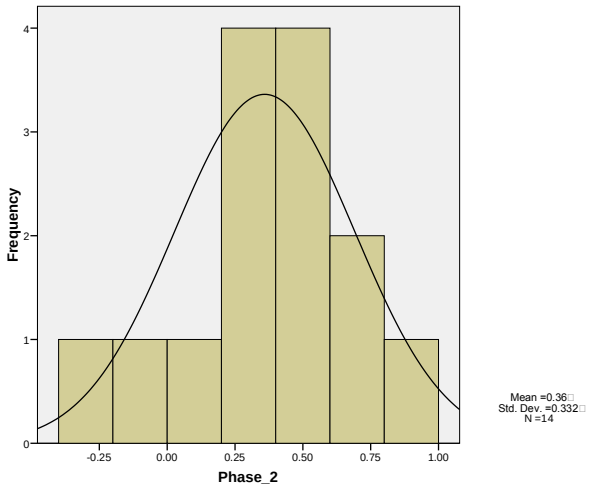

Fig. 5.2.2. Representative example of histogram of phase gradient data. The data is from a no-noise second trial.

APPENDIX 5.2

INDIVIDUAL SUPPRESSION AND PHASE GRADIENT DATA

Participant	CS_DP	CS_wave	CS_place	Phase	Phase_N
1	1.2	1.5	1.85	0.4	0.69
2	0.85	0.75	1.25	0.405	0.43
3	0.8	1	1.2	0.8	0.03
4	1.1	1	1	0.225	0.24
5	1.2	0.9	1.5	0.665	0.495
6	1.4	1.55	0.925	0.29	0.365
7	0.5	0.6	0.49	0.405	0.275
8	1	1.15	1.6	0.18	0.425
9	0.65	0.8	1.3	0.015	0.175
10	1.55	1.3	1.05	0.01	0.2
11	0.045	0.165	0.465	0.09	0.535
12	-0.1	-0.15	-0.9	0.495	0.21
13	0.85	0.55	2.15	0.525	0.48
14	1.95	0.95	1.9	0.715	0.565

(These data are from trial 1. Phase and Phase_N refers to phase gradient data without CAS and with CAS respectively).