High-Frequency Otoacoustic Emissions in Newborn Hearing Screening

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Abstract

Permanent congenital hearing loss is a common birth defect which has serious consequences for language, social and cognitive development. Newborn hearing screening permits early diagnosis and prompt treatment. Otoacoustic emission (OAE) testing provides sensitive and affordable newborn hearing screening but does not provide acceptable specificity because of transient middle-ear and external-ear conditions. OAE tests are usually performed for frequencies from 1 to 4 kHz. The present studies were undertaken to determine whether conducting OAE tests at higher frequencies might be effective in reducing false-positive rates. In study 1, a systematic review was conducted to evaluate the effects of different screening protocols on the outcome of the OAE test in the newborn population. Increasing the age at the OAE screening test, repeating the test and testing at higher frequencies were associated with lower referral rates. In study 2, the effects of middle-ear liquid on OAEs at different frequencies were investigated in chinchillas. The results showed significant reductions of OAE amplitudes across all frequencies, with more reduction for greater volumes of liquid. OAE noise-floor levels were increased at the lowest three frequencies following the introduction of middle-ear liquid, strongly affecting the signal-to-noise ratio. Study 3 was conducted to identify the sources of the increases in noise-floor levels when the middle ear contained liquid. Noise-floor measurements were carried out in animal experiments and artificial-cavity experiments. Noise-floor increases were greater with measurements taken in a non-sound-treated room than for those in a sound-treated room; greater with physiological endogenous noise than without; and greater with smaller cavity volumes. In study 4, high-frequency OAEs were assessed in newborns. We demonstrated the presence of OAEs in newborns up to 12 kHz and compared the outcomes of OAE tests at

these frequencies with the outcomes of the conventional OAE screening tests. OAEs at high frequencies resulted in greatly reduced false-positive rates. In addition, noise-floor levels were greater at 2 kHz in newborns who failed the conventional OAE test than in newborns who passed, but were not greater at high frequencies. Collectively these studies provide evidence for the advantage of OAE testing at high frequencies.

Résumé

La surdité congénitale est une condition commune qui pourrait mener à de graves conséquences pour l'acquisition du langage ainsi que du développement social et cognitif de l'enfant. Le dépistage diagnostic précoce des nouveau-nés permet un traitement rapide. Les mesures d'émissions oto-acoustiques (ÉOA) permettent un dépistage sensible et abordable de la surdité néonatale. En revanche, la spécificité de ce test n'est pas acceptable en raison de conditions affectant l'oreille moyenne et externe. Les tests ÉOA sont généralement effectués à des fréquences de 1 à 4 kHz. Notre étude a été menée afin de déterminer si des essais d'ÉOA effectués à de hautes fréquences pourraient réduire le taux de faux tests positifs. Dans l'étude 1, une revue systématique de la littérature a été menée afin d'évaluer les effets de différents protocoles de dépistage sur le résultat du test ÉOA dans la population des nouveau-nés. Le retard de l'âge du premier test de dépistage ÉOA, la répétition du test, et l'emploi de fréquences plus élevées se sont avérés utiles. Dans l'étude 2, nous avons étudié les effets des liquides contenus dans l'oreille moyenne sur les ÉOA à des fréquences différentes dans un modèle animal de chinchilla. Les résultats ont montré une réduction significative des amplitudes ÉOA pour toutes les fréquences, avec des réductions plus importantes quand les volumes de liquide augmentent. Suite à l'introduction de liquide dans l'oreille moyenne le bruit de fond des ÉOA augmente aux 3 fréquences les plus basses, affectant fortement le ratio signal-bruit. L'étude 3 a été menée pour identifier les sources de l'augmentation des niveaux de bruit de fond lorsque l'oreille moyenne contient un épanchement. Les mesures de bruit de fond ont été réalisées chez l'animal ainsi que dans une cavité artificielle. Les hausses du bruit de fond étaient augmentées : (i) dans une chambre non insonorisée au lieu d'une chambre insonorisée ; (ii) en présence de plus de bruit endogène physiologique; et (iii) avec des volumes de cavités plus petites. Dans l'étude 5, une

étude clinique évaluant l'effet des ÉOA à hautes fréquences a été réalisée chez des nouveau-nés. Nous avons démontré la présence d'ÉOA jusqu'à 12 kHz et comparé les résultats des tests à ces fréquences avec les résultats du test de dépistage conventionnel. L'ÉOA à des fréquences élevées réduit fortement les taux de faux - positifs. En outre, les niveaux de bruit de fond étaient plus élevés à 2 kHz chez les nouveau-nés qui ont échoué au test ÉOA conventionnel que chez les nouveau-nés qui ont réussi. Ceci n'est pas été observé à hautes fréquences. Collectivement, ces études fournissent des preuves des avantages d'utiliser le test ÉOA à des fréquences élevées.

Acknowledgements

First I would like to express my gratitude to Dr. Sam J Daniel, a dedicated and inspiring supervisor, for his commitment to the supervision of my work and for being a great mentor. He provided me with opportunities to travel to conferences and to supervise as well as teach students. These have been invaluable experiences for me. I equally thank my co-supervisor, Dr. W Robert J Funnell, for his painstaking supervision and guidance through my research and writing. My frequent meetings with Dr. Funnell were central to keeping me on track. I treasure the fact that I could always reach him over the phone to resolve urgent issues. I have learnt a lot from both my supervisors, who have been kind enough to have supported me with their training grants; I feel very privileged to have worked with both of them. I also appreciate my mentors in the IPN program, Dr. Hemant Paudel and Dr. Heidi McBride, for their support and for helpful advice on several occasions during my program. My thanks also go to the IPN program co-ordinators for all their support.

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I am grateful to my husband, Sola, who, since the beginning, has been by my side providing endless support and encouragement. I appreciate your presence, understanding and dedication. Special thanks also go to my lovely children, Ayooluwa Princess and Akinloluwa Oluwaseun, thank you for your understanding, you are my sunshine!

This dissertation is dedicated to God, who provided the strength and the inspiration, to my husband who is my encourager and cheerleader, and to my children, the sunshine that brightened the dark days.

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List of symbols and abbreviations

ABR – Auditory brainstem response

dB – Decibels

DP – Distortion product

ER – Energy reflectance

f – Frequency

FP – False positive

IHCs – Inner hair cells

JCIH – Joint Committee on Infant Hearing

HL – Hearing loss

Hz – Hertz

kHz – Kilohertz

ME – Middle ear

NIH – National Institute of Health

NS – Normal saline

NSTR – Non-sound-treated room

OAE – Otoacoustic emissions

PPV – Positive predictive value

PCHL– Permanent congenital hearing loss

SNR – Signal-to-noise ratio

STR – Sound-treated-room

UNHS – Universal newborn hearing screening

WBR – Wideband reflectance

Statement of original contributions to knowledge

The research studies in this dissertation provide the following original contributions to knowledge:

- First systematic review on the effects of different protocols on the referral and false-positive rates of the OAE test in the newborn period (Chapter 3).
- First study on the effects of amniotic fluid in the middle-ear on otoacoustic emissions in chinchillas (Chapter 4). Observations that the effects of middle-ear liquid on otoacoustic emissions at high frequencies are less than at low frequencies (Chapter 4).
- Measurement of the viscosity of human amniotic fluid and study of the effects of viscosity on OAEs. Observations of increases in the noise floors at low frequencies with the introduction of middle-ear liquid (Chapters 4 and 5) and with decreasing cavity volumes (Chapter 5).
- Distinguishing among the different sources of the noise-floor increase (Chapter 5).
- Observations of high-frequency OAEs in newborns up to at least 12 kHz (Chapter 6).
- Reduction of otoacoustic emissions referral and false-positive rates with high-frequency otoacoustic emissions test (Chapter 6).

Contributions of authors

Paper: *"Otoacoustic Emissions in Newborn Hearing Screening: A Systematic Review of the Effects of Different Protocols on Test Outcomes" by OV Akinpelu, E Peleva, WRJ Funnell, SJ Daniel*

(Chapter 3)

International Journal of Pediatric Otolaryngology; 2014 May;78(5):711–717

First author: Olubunmi V Akinpelu

Conceptualized the review and designed the search

Systematic review of literature and analysis of data

Drafted and reviewed the manuscript

Second author: Emilia Peleva

Systematic review of literature and analysis of data

Reviewed the manuscript

Third author: W Robert J Funnell

Supervised the review and critically reviewed the manuscript

Fourth author: Sam J Daniel

Supervised the review and critically reviewed the manuscript

Paper: *"Detection of Otoacoustic Emissions in Chinchilla when the Middle Ear contains Amniotic Fluid" by OV Akinpelu, WRJ Funnell, SJ Daniel*

(Chapter 4)

Accepted for publication in *Laryngoscope,* 2014 August 12

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Analyzed experimental data

Drafted and reviewed the manuscript

Second Author: W Robert J Funnell

Conceptualized and designed the experiments

Reviewed the manuscript

Third Author: Sam J Daniel

Conceptualized and designed the experiments

Reviewed the manuscript

Paper: *"Factors Determining Otoacoustic-Emission Noise-Floor Levels" by OV Akinpelu, WRJ Funnell, SJ Daniel*

(Chapter 5)

Manuscript in preparation

First author: Olubunmi V Akinpelu

Conceptualized and designed the experiments

Performed the experiments

Analyzed experimental data

Drafted and reviewed the manuscript

Second author: W Robert J Funnell

Conceptualized and designed the experiments

Reviewed the manuscript

Third author: Sam J Daniel

Conceptualized and designed the experiments

Reviewed the manuscript

Paper: *"High-Frequency Otoacoustic Emissions: A strategy to Reduce False-Positive Rates in Universal Newborn Hearing Screening" by OV Akinpelu, WRJ Funnell, S Daniel*

(Chapter 6)

Submitted to *Ear and Hearing*

First author: Olubunmi V Akinpelu

Conceptualized and designed the study

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Drafted and reviewed the manuscript

Second author: W Robert J Funnell

Conceptualized and designed the study

Reviewed the manuscript

Third author: Sam J Daniel

Conceptualized and designed the study

Reviewed the manuscript

CHAPTER 1 Introduction

Motivation

More than 40 years ago, the Joint Committee on Infant Hearing (JCIH) stated the importance of early recognition of permanent congenital hearing loss in newborns (JCIH, 1971). Initially, only children with known auditory risks were recommended for hearing screening (JCIH, 1971; 1982; 1990). However, about 50% of infants born with hearing loss have no known risk factors (NIH, 1993), so the committee later recommended that all newborns receive hearing screening, preferably during their hospital birth admission (JCIH, 1994). The goal of universal newborn hearing screening (UNHS) is the early detection of hearing loss and then intervention through integrated, interdisciplinary and family-centred intervention (JCIH, 2000). This has become the standard of care in many countries of the world.

UNHS seeks to conclusively diagnose infants with permanent congenital hearing loss by the 3rd month of life and commence treatment by the 6th month (JCIH, 2000). For these goals to be met, high sensitivity and specificity of the screening tests are crucial. Two methods have been employed to screen newborn hearing: automated otoacoustic emission (OAE) tests and automated auditory brainstem response (ABR) tests (e.g. Finitzo et al., 1998). Many programs employ a multi-stage hearing screening test whereby OAEs are used as the initial test; those failing the initial test are further screened with an automated ABR test. Newborns who fail the automated ABR test are referred for a more comprehensive diagnostic ABR test. Other programs refer newborns for diagnostic ABR following a single screening test with either the automated OAE or automated ABR test.

The ABR test has better specificity and sensitivity than the OAE test, but it takes a longer time to perform, requires the use of electrodes and specially trained personnel and is more costly (e.g., Kennedy et al.,1991). It also requires a longer duration of signal processing and averaging to extract the relevant signals from other brain activity, while little signal processing is needed to extract OAE signals from noise (Kemp, 2002).

The OAE test is non-invasive, less expensive, quicker and simpler to perform than the ABR test (e.g., Kennedy et al., 1991). However, unlike the ABR test where sound signals pass through the middle ear once, OAE detection relies on both forward and reverse transfer of sound signals. Acoustic stimuli that provoke the production of OAEs are transmitted forward to the inner ear via the external and middle ear, while the evoked OAEs are transmitted in the reverse direction via the middle ear to be detected in the ear canal. This makes the test critically dependent on the middle-ear status, so that departures from normal functioning of the middle ear have more impact on the OAEs than on the ABR. Thus, the major drawback of the OAE test is linked to its greater dependence on the middle and external ear.

As a result of this dependence, the OAE test has high false-positive rates, leading to an unacceptably high number of newborns being referred for complete audiological work up (e.g., Boone et al., 2005). The presence of amniotic fluid and mesenchyme in the middle ear of newborns has been strongly associated with such false positives (Chang et al., 1993; Thornton et al., 1993; Priner et al., 2003). Tympanic-membrane immobility, signifying the presence of middle-ear fluid, was found in 62.5% of newborns who failed the OAE screening test in one study (Doyle et al., 2000). It takes up to two days for amniotic fluid to drain out of the newborn middle-ear (Sade et al., 1976; Takahashi et al., 1992). Consequently, more newborns pass the test if it is repeated at later dates (Clemens et al., 2000; Clemens & Davis, 2001), but the logistics

and cost implications of a return visit to the hospital make this problematical. An average false-positive rate of about 8% is typical. Implementing a follow-up outpatient test has been shown to produce a reduced false-positive rate of about 1% by (Watkin 2001). Some authors have suggested delaying newborn hearing screening till the 20th day of life (e.g. Ng et al., 2004; Ghirri et al., 2011; Martines et al., 2012), while others have used multi-stage screening tests with both OAEs and the ABR test. These methods, however, have undesirable implications for cost and the time taken to perform the test. Therefore false-positive rates with OAE newborn hearing screening tests still remain a problem in UNHS.

It might be possible to address this problem by assessing the acoustical admittance of the middle ear to complement the OAE test in order to provide information on middle-ear status. However, as discussed in the next chapter, the nature of the external and middle ear of the newborn is quite different than in adults and makes admittance results difficult to interpret.

Rationale and objectives

The OAE test is potentially the ideal test for newborn hearing screening, given that it is inexpensive and easy to use. There is therefore a lot to gain if the problem of false positives is addressed. In this dissertation, I argue that OAE screening testing at high frequencies would result in reduced false-positive rates when compared with screening at low frequencies.

There are reasons to suggest that middle-ear disorders affect the transfer of high-frequency and low-frequency sounds differently (e.g., Nedzelnitsky, 1980; Gehr et al., 2004; Dalhoff et al., 2011). The amplitude of middle-ear vibration is greatest at a frequency called the resonance frequency. The resonance frequency of the middle ear is influenced by its stiffness and mass components. Increases in the stiffness components without changing the mass components lead to increases of the resonance frequency and decreases in the amplitudes of vibrations below the

resonance frequency, while increases in the mass components without changes in the stiffness components lead to decreases of the resonance frequency and decreases in the amplitudes of vibration above the resonance frequency. Very low frequencies are generally stiffness dominated while high frequencies are generally mass dominated (e.g. Silman & Silverman, 2000). The resonance frequency has been shown to be increased in clinical conditions associated with increased middle-ear stiffness, such as otitis media with effusion (Lai and Liu, 2008; Lai et al., 2008) and otosclerosis (Frade et al., 2000; Oguf et al., 2008). Increased middle-ear stiffness has also been shown to be associated with higher energy reflectance at low frequencies (Shahnaz et al., 2009).

The underlying hypothesis here is that OAEs at high frequencies will be sufficiently well transmitted through a middle ear that contains liquid (amniotic fluid), giving an acceptable signal-to-noise ratio, while at low frequencies the effect of middle-ear liquid will be more marked.

The overall aim of this study is to investigate the utility of high-frequency OAEs in reducing false-positive rates, particularly when they are due to middle-ear liquid. It is expected that results from this study will provide strategies to enhance the effectiveness of the OAE screening test, especially within the first 2 days of life, which corresponds to the usual time of newborn hearing screening (during the newborn's hospital birth admission).

Dissertation organization

This dissertation begins with a concise statement of the problem in this chapter. It continues with a description of the structure and function of the auditory system, and a discussion of hearing function, especially in relation to newborn hearing screening. This, in

addition to a review of the literature on middle-ear effects on otoacoustic emissions and the occurrence of false-positive results in newborn hearing screening, is presented in Chapter 2.

In order to understand the problems associated with the OAE screening test, a systematic review was completed as described in Chapter 3 of this dissertation. The effects of age at screening, the pass criteria, frequencies tested and the number of OAE tests performed on referral rates and false-positive rates were analysed. The chapter ends by recommending possible strategies for improving the OAE test which will be practicable during the hospital birth admission of the newborns.

Empirical studies were carried out with a chinchilla animal model which focused on the effects of middle-ear liquid on OAE detection at frequencies ranging from 825 to 13250 Hz. The specific objective of this study, as detailed in Chapter 4, was to evaluate the effects of four different volumes of liquid in the middle-ear (amniotic fluid and normal saline) on OAEs at both high and low frequencies and to correlate these with the effects on middle-ear energy reflectance at these frequencies. The effects of normal saline were studied in addition to those of amniotic fluid in order to determine whether the effects of the two liquids are different. There are only very scanty reports on OAE noise-floor changes with middle-ear anomalies. Therefore part of the work in this dissertation focused on studying the sources of OAE noise-floor changes occurring with middle-ear liquid; this is detailed in Chapter 5. The roles of the OAE equipment, physiological (endogenous) noise, testing environment (sound-treated versus non-sound-treated rooms) and artificial-cavity volume changes on OAE noise-floor changes were studied.

Furthermore, a study on high-frequency OAEs was carried out on newborns undergoing hearing screening, with the aim of determining the usefulness of high-frequency OAEs in

reducing referral and false-positive rates (Chapter 6). In this study the OAE test outcomes (pass/fail) in the newborns tested were evaluated frequency by frequency and examined in relation to the age at screening, the mode of delivery, the gestational age and birth weight.

Finally, Chapter 7 presents a summary of all the studies contained in this dissertation along with a discussion of the limitations and recommendations for future work.

CHAPTER 2 Background

This chapter is aimed at providing a broad introduction to newborn hearing screening. An overview of the anatomy and physiology of the hearing system is presented, with references to the peculiarities of the structure and function of the ear in the newborn period. This is followed by a brief description of hearing loss and hearing screening in the newborn period. A review of relevant previous studies is also presented.

Structure and function of the human auditory system

The auditory system is broadly divided into two parts: the peripheral auditory system and the central auditory system. The peripheral system includes the outer, middle and inner ear and the auditory nerve, while the central system includes structures from the brainstem to the auditory cortex. This review is largely based on Bear et al. (2007).

Figure 2-1 is an overview of the peripheral auditory system. The outer ear is made up of the auricle (pinna) and the external auditory canal. The auricle is made up of elastic cartilage and soft tissue covered with skin and is attached to the head by muscles and ligaments. The deep central portion of the pinna, known as the concha, leads into the external auditory canal, which in turn leads to the tympanic membrane. The external ear is separated from the middle ear by the tympanic membrane, a thin translucent oval-shaped membrane. The external auditory canal is S-shaped and is approximately 2.5 cm long in adults; its lateral one-third is cartilaginous while the inner two-thirds is bony.

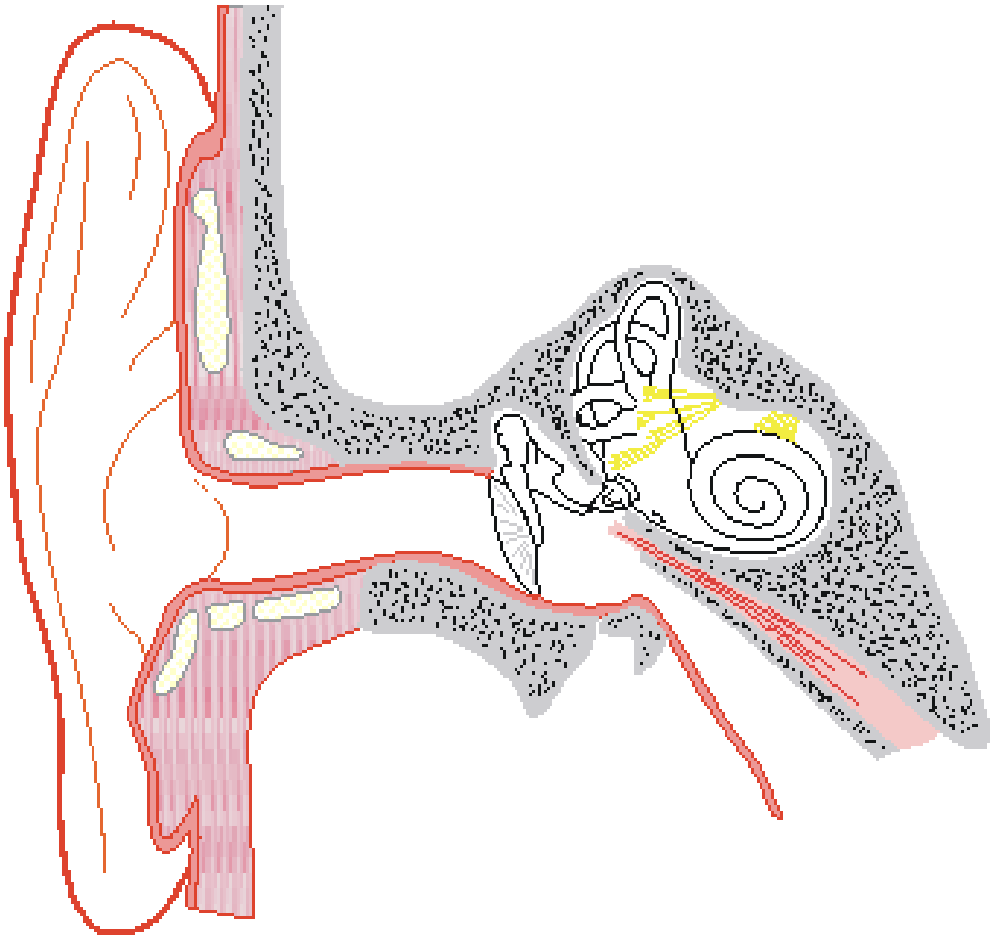

Figure 2-1: The peripheral auditory system. The system comprises the outer, middle and inner ear. (From http://audilab.bme.mcgill.ca/AudiLab/teach/me_saf/me_saf.html, accessed 2014 July 1, used with permission)

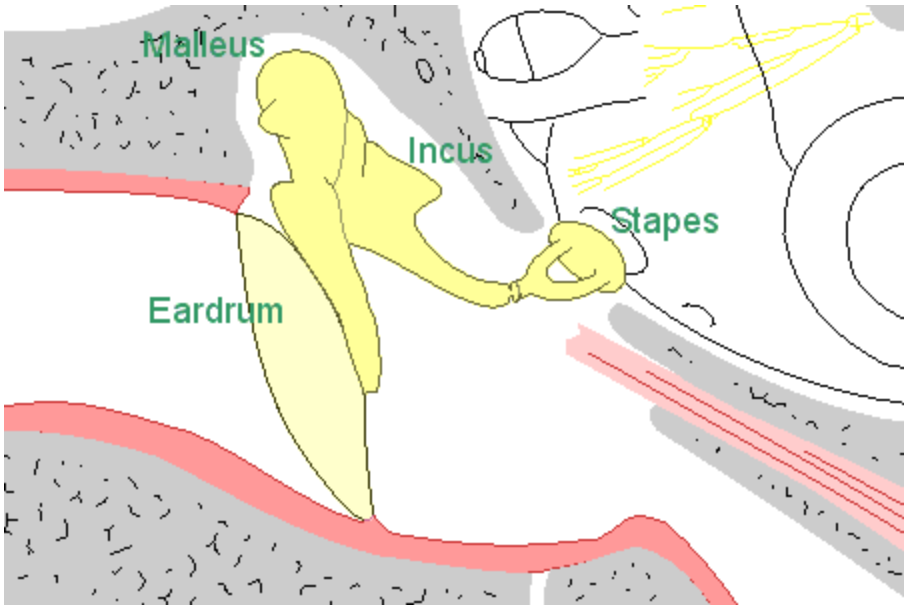

Figure 2-2: The tympanic cavity showing the tympanic membrane. (From http://audilab.bme.mcgill.ca/AudiLab/teach/me_saf/me_saf.html, accessed 2014 July 1, used with permission)

Figure 2-2 is a schematic overview of the middle ear, which is made up of the tympanic cavity, the mastoid cavity and the mastoid air cells (e.g., Donaldson et al., 1992; Bear et al., 2007). The middle-ear space typically has a volume of between 0.5 and 1 cm^3 (e.g. Gyo et al., 1986; Whittemore et al., 1998) while the mastoid air-cell system is usually between 1 and 21 cm^3 in volume (Molvaer et al., 1978; Koc et al., 2003). The tympanic cavity can be thought of as a six-sided space with a roof, a floor, anterior and posterior walls, and lateral and medial walls. The lateral wall is the tympanic membrane (eardrum), which is oval in shape and lies in the bony tympanic ring. The medial wall of the middle ear contains the promontory, which is the bulge of the basal turn of the cochlea. The roof is a thin plate of bone, the tegmen, while the floor is another plate of bone separating the middle ear from the jugular bulb. The anterior wall houses the Eustachian tube, while the posterior wall communicates with the mastoid air cells through the aditus.

The middle ear contains three small bones, known as the ear ossicles, which are named the malleus (hammer), incus (anvil) and stapes (stirrup), shown in Figure 2-3. It also contains the stapedius and tensor tympani muscles. The malleus is attached to the tympanic membrane through its handle (the manubrium), and is also connected with the incus in a saddle-shaped synovial joint. The incus in turn is connected with the stapes in a flexible synovial joint (e.g., Funnell et al., 2005). The base of the stapes, called the footplate, rests in the oval window and is fastened to it by the annular ligament.

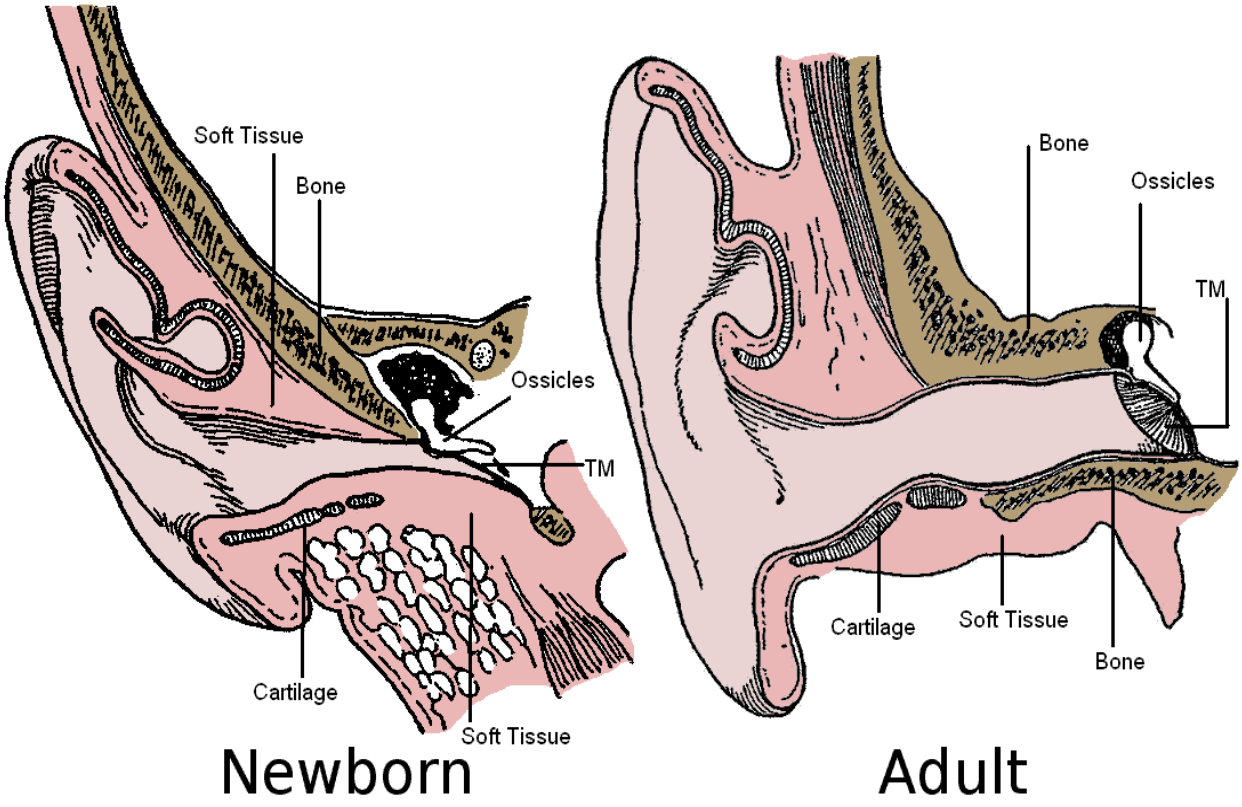

Figure 2-3: A comparison of newborn and adult ear canals. (After Fowler (1947), from http://audilab.bme.mcgill.ca/AudiLab/teach/me_saf/me_saf.html, used with permission)

Figure 2-3 shows the comparative anatomy of the newborn and adult external auditory canals and middle ears. In newborns, the ear canal is straight and is comprised mostly of cartilage and soft tissue, lacking the bony part except for the tympanic ring, which itself is not completely fused inferiorly (e.g. Fowler, 1947; Anson & Donaldson, 1981). In the course of the first year of life the tympanic ring continues to grow laterally until adolescence, and the ear canal continues to mature up to the 7th year of life with changes in the canal wall, the canal diameter and the position of the TM (Saunders et al., 1983; Northern & Downs 2002). The anatomical peculiarities of the newborn ear canal have implications for newborn hearing screening. Due to the fact that newborn ear canals are frequently collapsed, there is obstruction to the flow of sounds, which may produce false-positive test results (e.g., Hosford-Dunn et al., 1983; Vohr et al., 1996). In addition, the lack of ossification of the ear canal causes it to change volume in response to large static pressures, which in turn makes middle-ear admittance measurements difficult to interpret in the newborn (e.g., Qi et al., 2006). Another difference from adults is that the newborn tympanic membrane lies more horizontally in relation to the long axis of the ear canal (Ikui et al., 1997) as seen in Figure 2-3. The volume of the middle-ear space is smaller in newborns than in adults and continues to increase post-natally. The middle-ear volume is approximately 330 mm^3 at 22 days of age, 452 mm^3 at 3 months and about 640 mm^3 in adults, excluding the mastoid air cells (Ikui et al., 2000; Qi et al., 2006). A smaller middle-ear cavity volume implies increased middle-ear stiffness, because the membrane motion drives against the reduced volume compliance (Abdalla & Keefe, 2011). The ongoing maturation of the middle-ear system in the first few months of life gives rise to large changes in middle-ear function (e.g., Holte et al., 1991; Keefe & Levi, 1996; Sanford & Feeny, 2008).

The inner ear is made up of both the auditory and vestibular systems, which are found in the petrous part of the temporal bone (Figure 2-4). The auditory part is the snail-shaped cochlea which has two and a half turns comprising the apical, middle and basal turns. If uncoiled it would have a length of about 3.0 to 3.5 cm (Bear et al., 2007). The cochlea is tonotopically organized with low-frequency responses primarily in the apical turn and high frequencies at the base. It contains three fluid-filled compartments, namely the scala vestbuli, scala tympani and scala media, demarcated by the Reissner's membrane and basilar membrane respectively. The cochlea contains the organ of Corti, the outer wall of which includes the stria vascularis, and the spiral ligament, as well as the spiral ganglion cells.

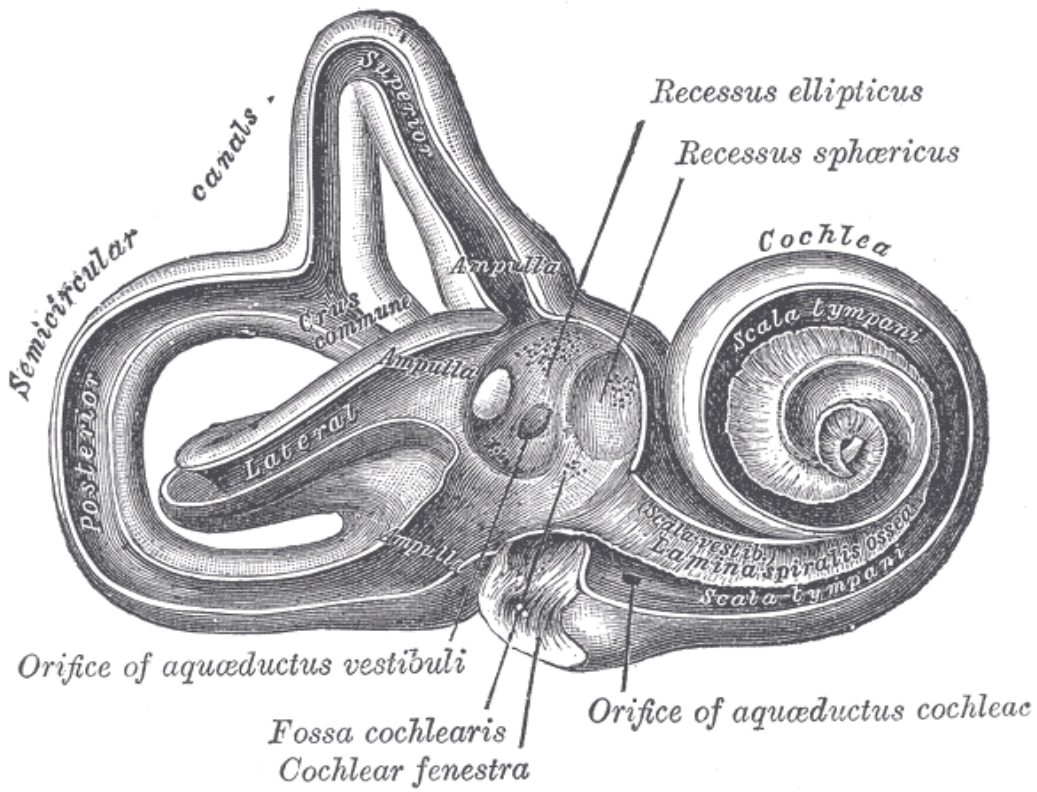

Figure 2-4: Auditory and vestibular systems in the inner ear. (From Gray's Anatomy, 1918, public domain)

Figure 2-5 is a schematic diagram of the organ of Corti showing the outer hair cells (OHCs), inner hair cells (IHCs) and the different supporting cells resting on the basilar membrane. The hair cells are so called because they have tufts of stereocilia (also called hair bundles) projecting from their surfaces.

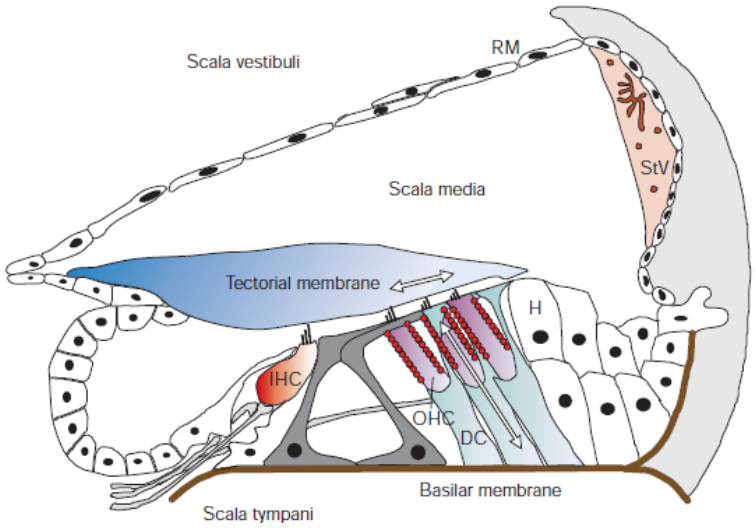

Figure 2-5: Schematic diagram of the organ of Corti. The stria vascularis occupies the lateral wall of the scala media and the three rows of outer hair cells (OHC) with the inner hair cells (IHC) and supporting cells (DC=Deiters' cells, H=Hensen's cells) sit on the basilar membrane. (From Ashmore et al., 1999, used with permission)

Figure 2-6 shows the neuroanatomical pathway in the central auditory system, which begins with the auditory nerve fibres travelling from the cochlea via the brainstem to the auditory cortex. The pathway includes the auditory brainstem (the cochlea nucleus, the trapezoid body, the superior olivary complex and the lateral lemniscus); the midbrain (the inferior colliculi); the thalamus (the medial geniculate nucleus) and the auditory part of the cerebral cortex. The first synaptic connection of the auditory pathway is at the cochlear nucleus, located in the dorsolateral side of the brainstem. The axons of neurons from the cochlear nuclei from the right and left sides proceed to the superior olivary complex in the medulla, which is the first place of binaural

convergence of auditory neurons. From this complex, the neuronal axons proceed to the inferior colliculus in the midbrain. The outputs from here then continue to the medial geniculate body, also known as the auditory thalamus, from where the outputs are finally sent to the auditory cortex.

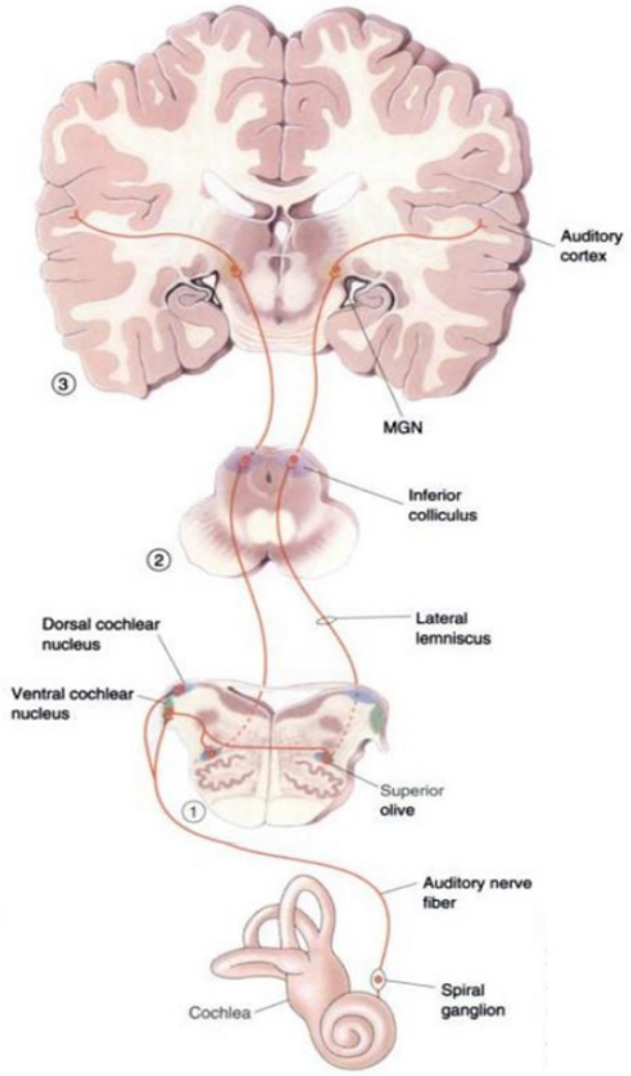

Figure 2-6: Central auditory pathways. This is a schematic diagram of the primary pathways of auditory signals from the spiral ganglion in the cochlea to the auditory cortex via the medial geniculate nucleus (MGN) of the thalamus. (From Bear et al., 2007, used with permission).

Development of the auditory system

The development of the external ear begins in the 4th and 5th week of the embryonic period and becomes well organized by the 20th week-yes (Wright 1997; McPhee & Van De Water, 1988). The different middle-ear components arise from different embryonic origins. At the 8th week, the primordial malleus, the developing tensor tympani muscle and the developing auditory tube are visible. The different components of the ossicles are in place by the 28th week, while other middle-ear structures are fully formed structurally by the 31st week. However, growth and development of the middle ear and mastoid process continue until puberty. During embryonic development, the auditory placode is the first structure of the inner ear to be seen, by the 4th week, becoming the otocyst by the 5th week. From the otocyst develop the structures of the inner ear, including the cochlear duct. The cochlear duct reaches its final extent of two and a half turns by the 11th to 12th week. The differentiation of the hair cells in the cochlea begins between the 10th and 12th weeks (Hall 2000; Kandell et al., 2000; Counter 2010). The neurosensory part of the auditory system begins to develop after the 20th week. By the 24th week, the auditory system is structurally complete. The entire auditory system becomes functional at around the 25th to 29th week when the ganglion cells of the spiral nucleus in the cochlea connect inner hair cells to the brainstem.

Physiology of hearing

Properties of sound

Sound is composed of vibrations that propagate as mechanical waves of pressure and displacement, typically through the air. Its characteristics include frequency, wavelength, wave number, amplitude and sound pressure (Figure 2-7). Frequency is the number of waves of sound occurring per unit time, and has the unit hertz (Hz). One hertz corresponds to one cycle per

second. The wavelength of a sound wave is the spatial period of the wave. It has an inverse relationship with frequency:

$$f = \frac{v}{\lambda}$$

where v is the velocity of sound, λ is the wavelength and f is the frequency.

All sounds are propagated by periodic rarefaction and compression of the medium (Figure 2-7).

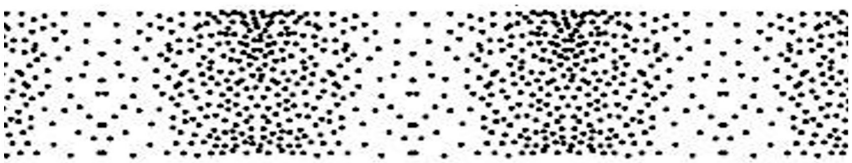

Figure 2-7: Properties of sound. This figure illustrates rarefaction and compression of the medium propagating the sound. (Courtesy of Farid Ibrahim)

Human ears are able to perceive sounds of frequencies between 20 and 20,000 Hz. Although most speech sounds range between 200 and 8,000 Hz, human ears are most sensitive to sounds at lower frequencies (e.g., Faslt & Zwicker, 2007). The frequency range of hearing varies with age, occupational exposure and gender. High-frequency hearing is diminished with increasing age: most hearing at 20000 Hz is lost by the teenage years, and in the elderly population the hearing at frequencies above 4000 Hz is often impaired.

Roles of external, middle and inner ear in hearing function

The function of the ear canal is that of propagation of sound pressure waves along its length to the tympanic membrane and amplification of sounds of frequencies between 3 and 12 kHz (Ballachandra, 1995). The tympanic membrane acts as a transducer, transforming sound pressure into mechanical movement. The movement of the tympanic membrane causes the malleus and the incus to vibrate, which in turn moves the stapes back and forth in the oval window, setting up a wave of sound pressure in the fluid of the inner ear. The movement of the tympanic membrane varies at different frequencies: at low frequencies all of it moves in phase, while at higher frequencies the vibration pattern breaks into smaller portions that vibrate at different phases (Khanna & Tonndorf, 1972).

The middle ear is understood to improve the impedance match between air-borne sound waves arriving from the ear canal and the liquid medium of the inner ear, thus reducing the loss of acoustic energy that usually occurs when sound waves transit from air to a liquid. The combination of the ratio of the tympanic-membrane diameter to that of the oval window (approximately 23:1 in human), and the ratio of the length of the manubrium to that of the long process of the incus (1.3:1) is often said to produce an increased level of sound pressure at the oval window (e.g., Puria et al., 1997; Merchant et al., 1998). The situation is really more complex (e.g., Funnell, 1996). The actual pressure gain in tympano-ossicular coupling is frequency-dependent, being highest at approximately 1 kHz, the resonant frequency of the middle ear, and decreasing by 6 to 8 decibels per octave at higher frequencies (Puria et al., 1993; Kurokawa & Goode, 1995). There is hardly any gain above 7 kHz. A mean gain of 23 dB SPL was reported by Kurokawa & Goode (1995) below 1 kHz, with a mean peak gain of about 27 dB SPL.

The vibrating mechanical system of the middle ear, like any other vibrating system, comprises mass, stiffness and friction components. No middle-ear component is purely mass or purely stiffness. However, the main components contributing to mass include the ossicles and the perilymph, while the main components contributing to stiffness include the volumes of air in the outer-ear and middle-ear spaces, the tympanic membrane, the tendons and ligaments of the ossicles, and the incudomallear and incudostapedial joints. The inertial forces are produced by mass and acceleration, while stiffness forces are proportional to the deflections of spring-like structures from their resting positions (e.g., Avan et al., 2000). The vibrating system also includes frictional or damping forces which dissipate energy in the form of heat when movements of the constituent parts occur.

Following the mechanical transfer of sound from the middle ear, the inner hair cells perform the task of electro-transduction, which is the process of converting mechanical sound energy to electrical signals which are then transferred to the auditory nerve. The outer hair cells, on the other hand, perform the role of amplification of low-level sounds by the movement of their hair bundles and the electrically driven motility of their cell bodies. Figure 2-8 illustrates the function of the outer and inner hair cells (Yates et al., 1992). The basilar membrane of the organ of Corti moves in response to acoustic energy (shown as step 2 in Figure 2-8); this movement results in relative motion between the tectorial membrane and the reticular lamina which ultimately leads to the displacement of hair bundles and changes in their receptor potentials (step 3). This in turn leads to the production of mechanical force (step 4) that is fed back to produce more basilar-membrane movement (step 5). The contraction and elongation of OHCs is non-linear and is the basis for the non-linearity in cochlear amplification (Kemp, 1978; Robinette & Glattke, 2002). This contraction and elongation of the outer hair cells in response to

their own generated electricity is what is thought to give rise to otoacoustic emissions (Brownell, 1990; Nuttall & Ren, 1995).

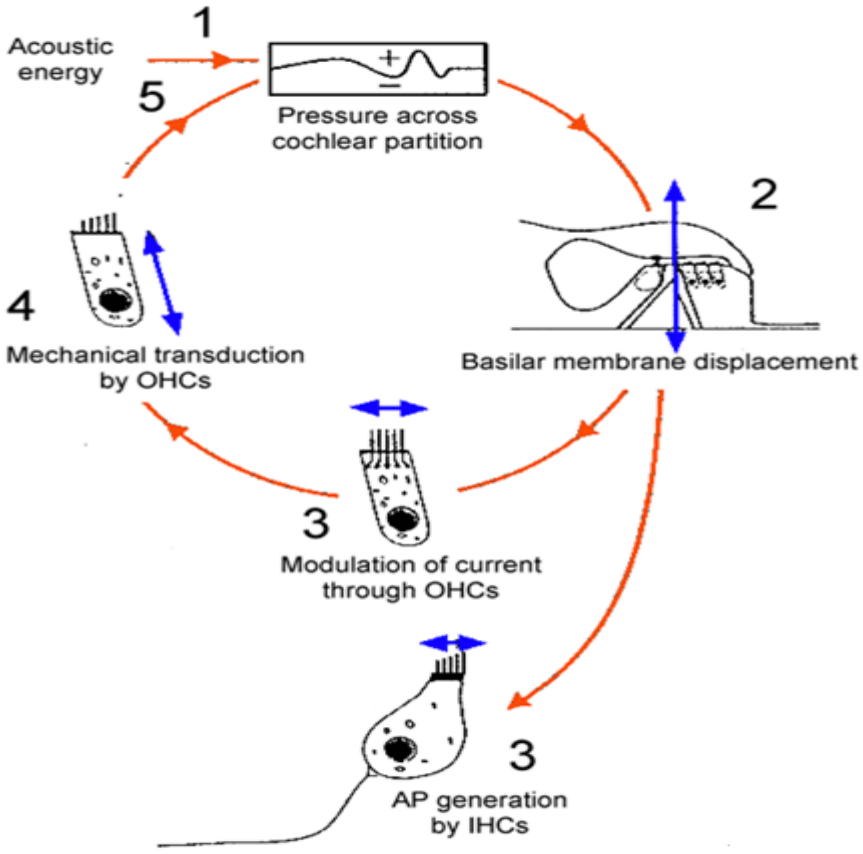

Figure 2-8: Active processes within the cochlea. (1) Vibration of the basilar membrane in response to acoustic stimulation. (2) Modulation of the inner and outer hair cells as a result of the shearing between the tectorial membrane and the tops of the hair cells. (3) Receptor potential is generated within the outer hair cells, which (4) then triggers some mechanical activity in the outer hair cell. (5) The motility of the outer hair cells provides feedback pressure fluctuations into the scala vestibuli to complete the loop. The receptor potential within the inner hair cell leads to transmitter release across the afferent synapse. (From Yates et al., 1992, used with permission).

Assessment of hearing function

Testing for hearing function is important for identification of hearing loss, either for screening or diagnostic purposes. This section is based largely on ASHA (1997) and Gelfand (2011). Hearing tests are categorized into two broad divisions, namely, behavioural and objective. Behavioural tests require the cooperation of the participant. They include pure-tone audiometry and speech-discrimination tests, both of which assess the entire auditory system and are often the first tests carried out in adults and older children to evaluate hearing loss. Play audiometry is suitable for children between the ages of 3 and 7 years. For infants between the ages of 7 to 8 months and 3 years, visual-reinforcement orientation audiometry can be used to test hearing function in a free field. The child is conditioned to turn the head when he or she hears sounds. Behavioural observation audiometry is suitable for infants less than 7 months of age. In this test the infant is observed for changes in behaviour in response to sound.

Objective hearing tests, on the other hand, do not require active cooperation from the participant and for this reason they are suitable for hearing assessment in newborns. They include middle-ear tests, ABR, and otoacoustic emissions. Since the main focus of this work is on otoacoustic emissions, middle-ear tests and ABR will only be described briefly.

Tests of middle-ear function

Middle-ear status can be assessed with tympanometry, which is the measurement of the input acoustic immittance of the ear as a function of ear-canal pressure (e.g., Katz 2002). Immittance is a generic term that includes impedance and admittance. Input impedance (Z) is a measure of how difficult it is for sound to make the middle-ear system vibrate, while input admittance (Y) is the reciprocal, a measure of the ease with which sound makes the middle-ear system vibrate. Admittance is expressed as U/P where U = volume velocity and P = sound

pressure. Admittance is determined by the compliance, mass and friction parts of the middle ear. Admittance is expressed as a 'complex' value with 'real' and 'imaginary' components, with the real component (conductance) being determined by the friction (which involves the dissipation of energy) and the imaginary component (susceptance) being determined by the compliance and mass parts of the system.

Clinical measurement of acoustic immittance involves inserting an earpiece (probe tip) into the ear canal. The probe tip contains four tubes: the first tube is for the loudspeaker which is used to deliver a probe tone into the ear canal; the second tube is for the microphone used to monitor the probe sound level in the ear canal; the third tube connects to the pressure pump and manometer; and the fourth tube connects to a reflex loudspeaker. The reflex loudspeaker is specifically involved with acoustic-reflex testing, which measures the response of the middle-ear system to intense sounds and which is not of concern in the current study. Most routine tests use a 226-Hz probe tone. The loudspeaker generates the volume velocity while the microphone measures the resulting sound pressure in the ear canal. The admittance is a measure of the middle-ear status (e.g., Silman & Silverman, 2000). Tympanometry with a 226-Hz probe tone gives rise to misleading results in the first 6 months of life; as a result of this, high-frequency tympanometry has been used in newborns and young infants (e.g., Kei et al., 2003).

Wideband energy reflectance (WBR) is a relatively recent addition to the tests for middle-ear status. Energy reflectance is the ratio of the acoustic energy reflected from the tympanic membrane to the energy that strikes it, and in wideband measurements it is assessed across a range of frequencies. A probe is placed in the ear canal and a wideband chirp or click stimulus is delivered, with the resulting energy-reflectance values plotted as a function of frequency. Energy reflectance can be calculated from the input admittance.

Studies have shown that wideband energy reflectance measurements are repeatable in newborns (e.g., Keefe et al., 2000, 2003). It has therefore been suggested that the addition of energy-reflectance measurement as part of the battery of tests for newborn hearing screening has the potential for providing differential diagnosis of hearing loss in newborns (Keefe et al., 2000). Wideband energy reflectance was employed in this research because its wide range of frequencies allows for comparisons with OAEs over the range of frequencies that are of interest here.

Auditory brainstem response

The auditory brainstem response (ABR), a series of electrical potentials recorded on the scalp, is a measure of auditory synchrony along the auditory pathway. It is an evoked response arising from the auditory pathway through the cochlear nucleus, the lateral lemniscus, the olivary nucleus, the lateral lemniscus, and the inferior colliculi. The ABR test is the most common form of electrophysiological hearing testing. The six waves of the ABR correspond to the different origins of the response (Figure 2-9) and have been used not only to test the functional status of the central auditory pathway but also to determine the threshold of hearing (e.g. Probst et al., 2006; Bear et al., 2007).

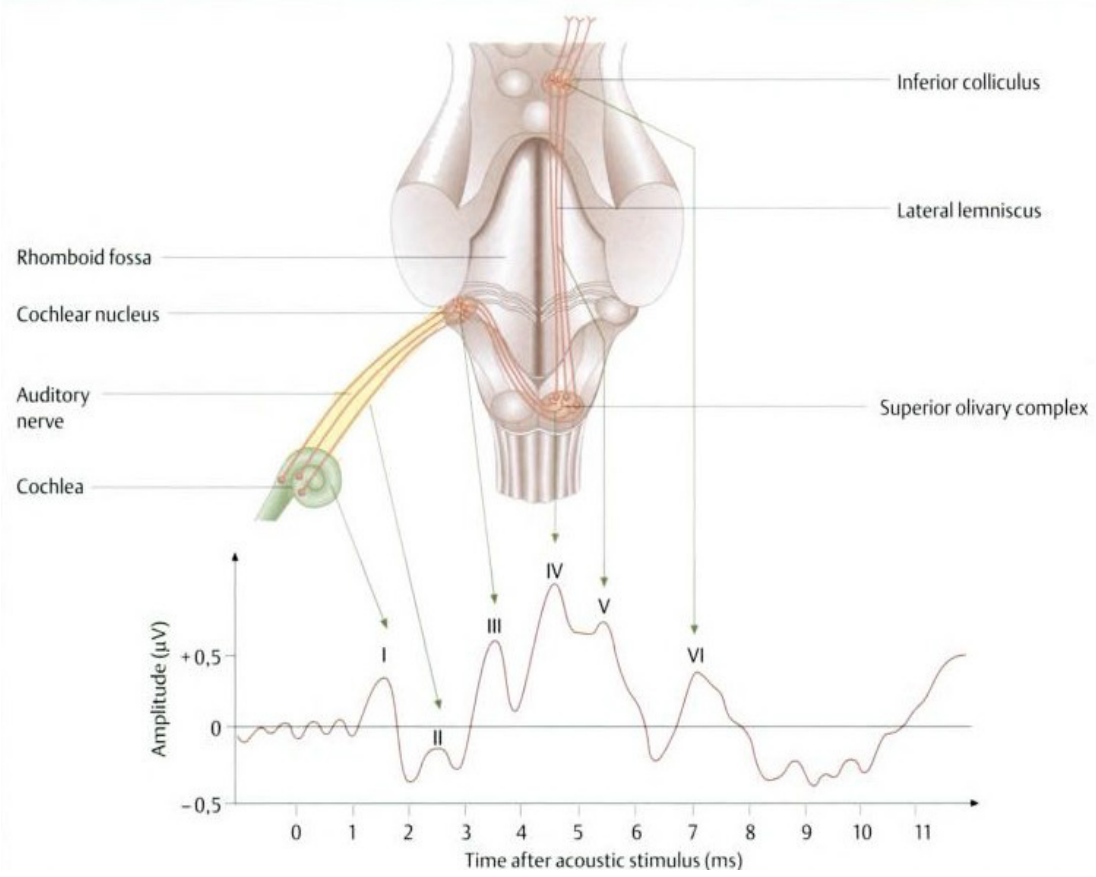

Figure 2-9: Diagram of the auditory brainstem response. Anatomical sites of the different waves are shown; (From Probst et al., 2005; used with permission)

Failure at the automated screening ABR test does not necessarily mean that there is hearing loss, so a diagnostic ABR test is performed on all newborns who fail the screening test.The ABR is present from the 25th week of gestation (Starr et al., 1977). Because the ABR test requires little or no cooperation from the subject, it is suitable for assessing hearing in newborns and has been in use since the 1980's as a tool for screening hearing in high-risk newborns (JCIH, 1982). Currently, an automated version is used in most universal newborn hearing screening (UNHS) programs. The Automated ABR test makes use of broadband stimuli (at 30-40 dB SPL intensity) which evoke a response wave-form that is then matched to a template by an algorithm within the screening device. The congruence of the response to the

template is used to determine the pass or fail status of the newborn. The diagnostic ABR test confirms the presence of hearing loss and is able to give the threshold for hearing at specific frequencies, which is required before definitive treatment can commence.

Otoacoustic emissions

Otoacoustic emissions (OAEs) are low-level sounds that are produced by a normal-functioning cochlea, first observed by Kemp (1978). The sounds are transmitted backwards through the middle ear and can be recorded with a microphone in the ear canal. It is now known that OAEs are generated from two distinct mechanisms: an active non-linear component involving the OHCs, and passive linear reflection of the travelling wave along the basilar membrane (Ashmore, 1987; Evans et al., 1989).

There are two basic categories of OAEs: spontaneous and evoked (Probst, 1990). Spontaneous OAEs occur without external stimulation. They are typically highly stable pure tones at about −10 to +30 dB SPL and are present in 30 to 40% of healthy young ears (Burns et al., 1992; Penner & Zhang, 1997). They have limited clinical use since they are not measurable in all ears and occur at discrete, unpredictable frequencies. Evoked OAEs occur in response to external stimulation. The stimuli that are commonly used for evoking OAEs are of two different types: clicks or tone bursts which generate transient evoked OAEs (TEOAEs), and pairs of primary tones (f_1, the lower-frequency pure tone, and f_2, the higher-frequency pure tone) which generate pure-tone distortion product OAEs (DPOAEs) due to the non-linearities in the cochlea (e.g., Kemp 2002). The distortion products occur at frequencies (f_{dp}) that are sums (or differences) of multiples of the primary-tone frequencies. The strongest and the most commonly used f_{dp} is the $2f_1$-f_2 product. DPOAEs have been shown to be diminished or eliminated at the frequencies

which correspond to the region of hearing loss and as such are able to give better frequency-specific assessment than TEOAEs.

The ratio of the intensity of the detected OAE response to the intensity of the noise measured in the canal is the signal-to-noise ratio (SNR), which is used in many contexts to determine the presence or absence of OAEs in neonatal ears (Brass & Kemp, 1994).

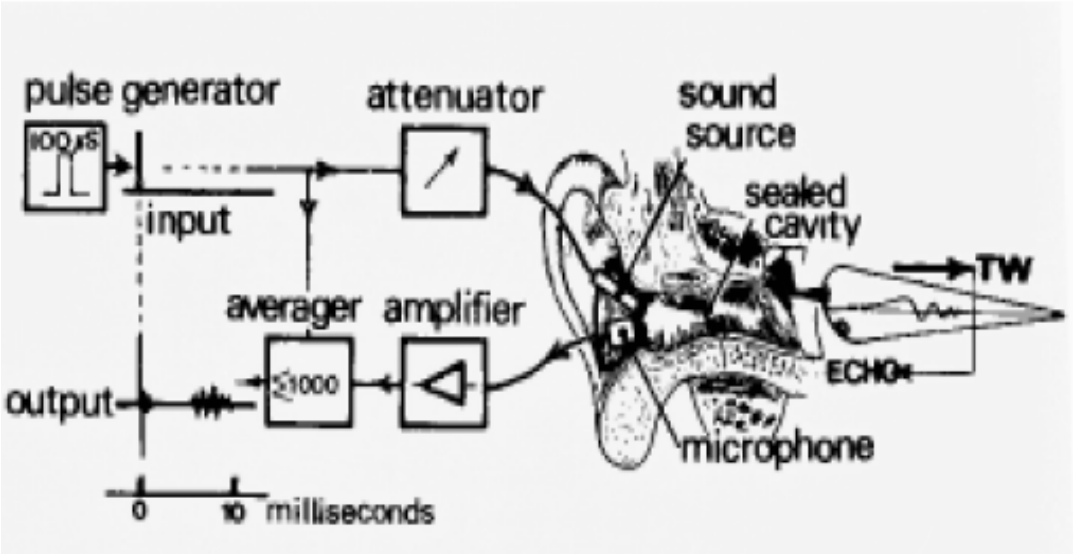

Figure 2-10: A schematic diagram of OAE testing. The sound stimulus traverses the outer and middle ear to reach the cochlea where it generates a travelling wave (TW) and resultant otoacoustic emissions (ECHO) which travels backwards through the middle ear to the outer ear where it is detected. (From Otodynamics; used with permission)

Figure 2-10 shows the set-up of the OAE test. The test is conducted by inserting the OAE probe, which has a soft flexible tip, in the ear canal to obtain a seal. The probe contains a miniaturized loudspeaker and a microphone. An adequate ear-canal seal with the probe is essential to exclude external sounds.

OAE spectra are computed with the fast Fourier transform (FFT), which is a mathematical algorithm employed to transform signals between the time domain and the frequency domain (e.g., Jansenn & Muller, 2008). The OAE equipment used for hearing screening is automated to generate pass or fail results based on preset criteria that involve an acceptable SNR value for a given number of frequencies. Most equipment accepts an SNR of between 3 and 7 dB at 3 out of 4 tested frequencies for a pass (e.g., Oudesluys-Murphy et al., 1991; Doyle et al., 1997).

Noise floors are defined as noise levels below which signals cannot be detected. All OAEs are analyzed relative to the noise floor. The noise-floors originate from body sounds as well as from external sources, which include probe tip movement and equipment noise (e.g., Arlinger, 1981). Noise floors are usually higher at low frequencies, in part because ambient noise and body sounds are often made up of low frequencies. Different methods have been used to measure noise-floor levels and to separate signals from noise. Different signal-processing approaches have been used for TEOAEs (e.g., Giebel, 2001): one is the calculation of the correlation between two subsets of averaged data, another is the calculation of the spectral power ratio of the sum and the difference of the two different subsets, and a third is based on a binomial statistic calculation of the probability that an emission has occurred. On the other hand, the noise-floor amplitude in DPOAEs is calculated by averaging the signal amplitudes present at several frequencies near the DP frequency. DPOAEs can be separated from the noise by calculating the SNR from the DP level measured in the DP FFT bin in comparison to the levels in the closely adjacent-frequency bins which contains only background noise.

Reduction of the noise-floor levels is undoubtedly important to successful recording of OAEs in the ear canal. Probe noise attenuation and signal averaging are methods that have been

used to reduce the noise-floor levels (Berger & Killion, 1989). Beattie et al. (2000) showed that increasing the number of sweeps and averaging was helpful in reducing non-synchronous noise-floor levels. Delgado et al. (2000) used a time-domain adaptive noise-cancellation method to estimate and eliminate noise from signals and showed the usefulness of this in DPOAE recordings.

Noise-floor levels tend to be higher in infants than in adults, but follow the same pattern of being smaller at higher frequencies (Gorga et al., 2000). The reduction of ambient acoustic noise has been shown to improve the outcome of newborn OAE test (Lin et al., 2007; Olusanya, 2010; Salina et al., 2010).

Hearing and deafness in newborns

Hearing loss is a highly prevalent congenital condition occurring in about 2 to 3 per 1000 live births (Vohr, 2003). It is considered to be the most common birth defect, much more common than diseases like congenital hypothyroidism (30 per 100 000), phenylketonuria (10 per 100 000) and galactosemia (2 per 100 000 live births) (Committee of Genetics, 1996; Mayatepek et al., 2010). In general, hearing loss is classified as conductive, sensorineural or mixed, depending on the site of the pathology in the auditory system. Conductive hearing loss results from abnormalities of the external and/or middle ear, while sensorineural hearing loss results from abnormalities of the cochlea, the auditory nerve and/or the central processing centres. Hearing loss is also categorized based on the onset of the hearing loss into congenital and acquired hearing loss. Congenital hearing loss is that which is present at birth. About 50% of cases of congenital hearing loss are due to genetic causes (Smith et al., 2008; Kover et al., 2011), and about 20% of these are syndromic (Morton & Nance., 2006). Non-hereditary causes of hearing loss include pre-natal infections in about 30% of newborns with hearing loss (Morton &

Nance 2006; Grosse et al., 2008; Brown et al., 2009); prenatal exposure to toxins; and peri-partum conditions like anoxia and hypoxia (e.g., Fligor et al., 2005), hyperbilirubinemia (e.g., Akinpelu et al., 2013) and rhesus isoimmunization. Low birth weight (e.g., Van Naarden & Decoufle, 1999) and prematurity are also associated with perinatal hearing loss. Congenital hearing loss is usually of the sensorineural type. Conductive congenital hearing loss is typically associated with congenital malformations of the external and/or middle ear and with fluid in the middle ear.

Undiagnosed hearing loss leads to delayed speech, language and cognitive development. For example, the language ability of early-identified newborns is superior to that of their late-identified counterparts even when their treatments are identical (Yoshinaga-Itano et al., 1998; Yoshinaga-Itano & Gravel, 2001). Early diagnosis and appropriate and timely intervention are therefore critical. Hence, the goal of UNHS is to identify newborns with possible hearing loss within the first month of life. The two screening methods that have been used to achieve this goal are the automated OAE test and the automated ABR test; different protocols have been employed in different institutions using either OAE or ABR or both. The ABR test has lower fail rates than the OAE test (Vohr et al., 2001) and will aid in detecting neural hearing loss but misses mild cases of hearing loss (Johnson et al., 2005) and has higher costs of disposables and a greater test time than the OAE test (Vohr et al., 2001). The sensitivity of the ABR test for detecting hearing loss is between 45 and 100% using behavioural audiometric testing as the gold standard (e.g., Shimizu et al., 1990; Smyth et al., 1990; Stevens et al., 1990), while the specificity is between 71 and 99% (e.g. Durieux-Smith et al., 1991; Savio et al., 2006). The OAE test misses neural hearing losses, but this type of hearing loss has a very low incidence in healthy newborns (Berg et al., 2011) so this is not a great disadvantage.

OAE newborn hearing screening and the middle ear

OAE testing alone has been used in newborn hearing screening for more than 2 decades (White, 2003). Referral rates between 6.5 and 13% have been reported for tests conducted during the hospital birth admission (Vohr et al., 1998; 2001; Watkin, 1996). With behavioural tests as the gold standard, the sensitivity of the newborn OAE test is between 55 and 100% and the specificity is between 71 and 91% (e.g., Stevens et al., 1990; Apostolopoulos et al., 1999; Roth et al., 2008; Meena et al., 2013). Owing to the requirement of a normal middle-ear function for the OAE test (e.g., Robinette & Glattke 2002), it is understandable that the high referral rates and low specificity seen with the newborn OAE test have been connected to transient abnormal middle-ear conditions in the newborn. Evidence in support of this is the fact that newborns who do not pass the OAE screening test at birth also have decreased middle-ear absorbance for frequencies between 1 and 3 kHz (Hunter et al., 2010; 2013; 2014), which is similar to what is found in children with confirmed middle-ear effusion. Lehman et al. (2008) showed in a cohort of Australian Aboriginal children that the absence of OAEs was predictive of otitis media. Likewise, Shahnaz et al. (2008) showed the outcome of tympanometry at 1 kHz to be a good predictor of the presence or absence of OAE in newborns. These findings are buttressed by previously reported effects of middle-ear pathologies on OAEs in older children (e.g., Trine et al., 1993; Amedee, 1995; Avan et al., 2000; Tas et al., 2004). Consequently, it has been suggested that a middle-ear evaluation test be included in newborn hearing screening so as to provide better interpretation of hearing-screening results. High-frequency tympanometry and wideband acoustic immitance measurements in the newborns are currently being studied by various researchers as potential components of the newborn hearing screening test battery (e.g., Hunter et al., 2010; Kilic et al., 2012; Aithal et al., 2013). However, the evidence backing the diagnostic accuracy of these tests in newborns is still limited.

The striking dependence of the OAE test on normal middle-ear function not only has an undesirable effect on OAE test outcomes but also suggests the possibility of utilizing OAEs to indirectly assess middle-ear function. The OAEs detected in the ear canal are shaped by their passage through the middle ear and may potentially provide useful information about the status of the middle ear. In a very early study, Nozza et al. (1997) studied 66 children classified into pass/fail by pure-tone hearing tests and otoscopy. In their study, OAE testing was found to perform well in identifying ears with middle-ear disease. However, the correlations between middle-ear immittance measurements and OAE variables were low. Further studies have been used to relate middle-ear assessment with OAEs. Janssen et al (2005) proposed the use of DPOAEs to differentiate between middle-ear and cochlear disorders in newborns. Experimental studies carried out a few years later showed that umbo-vibration distortion product OAEs could be used to measure the reverse transmission through the middle ear in gerbils with a potential application for differentiating middle-ear pathologies from cochlear pathologies (Turcanu et al., 2009; Dalhoff et al., 2011). In a related study, Olzowy et al. (2010) proposed a quantitative method of estimating conductive hearing loss with the use of distortion product OAEs: given that conductive hearing loss would attenuate L_1 and L_2 of the primary tones f_1 and f_2, it may be possible to estimate conductive hearing loss by determining the change in L_1 that is required to restore OHC excitation and produce maximal OAE level. However, the utility of OAEs in this context has yet to be validated.

The middle-ear transfer function is frequency specific. Likewise, the mechanics of the middle ear when it contains liquid differs in a frequency-dependent fashion from when there is no liquid. The presence of liquid in the middle ear reduces the effective volume of the middle-ear air space, which reduces middle-ear compliance and leads to a reduction in vibrations at low

frequencies (Ravicz et al., 2004). Following induced otitis media with effusion in a guinea-pig animal model, the observed reduction in tympanic-membrane and round-window-membrane mobility was less at high frequencies (Dai & Gan, 2008). Similarly, conditions leading to increased middle-ear pressure produced decreased movement of the tympanic membrane mainly at frequencies below 1 kHz (Murakami et al., 1997; Gan et al., 2006).

Likewise, frequency-specific patterns have been shown in newborn-hearing tests. As mentioned above, newborns who failed initial OAE tests had reductions in middle-ear absorbance mainly for frequencies 1 to 3 kHz (e.g., Hunter et al., 2013). One wonders then if the same frequencies would be affected in OAE tests. A study comparing OAEs at low and high frequencies, particularly with reference to newborn hearing screening, is required in order to gain further understanding of OAEs in the group of newborns who are falsely referred.

Otoacoustic emissions and false positives

The occurrence of a "refer" screening outcome in a newborn that has normal hearing is defined as a false-positive result (Figure 2-11). The false-positive rate is therefore defined as the proportion of normally hearing newborns who are referred for diagnostic testing (e.g., Patel & Feldman 2011). In spite of continued improvement in OAE technology, false positives are still a problem (Torkaman et al., 2012; Ulusoy et al., 2013). Rates as high as 13% have been reported (Olusanya, 2010), some of which have been related to middle-ear issues (Xu et al., 2011).

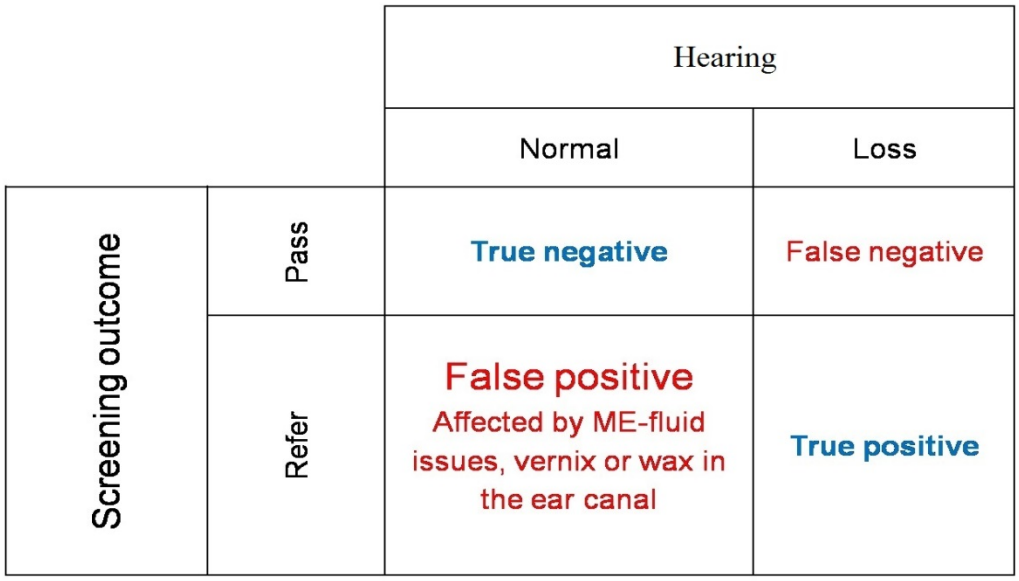

Figure 2-11: Hearing screening outcomes.

False-positive results and their associated problems merit attention in both clinical practice and research. False-positive results lead to parental anxiety and affect the parent-newborn relationship. About 50% of mothers of babies with positive hearing screening results reported emotional disturbances like anger, confusion, depression, frustration, shock and sadness (Weichbold & Welzl-Mueller, 2001), and for a significant number of mothers the concern was ongoing (Poulakis et al., 2003). False-positive results also lead to unnecessary follow up which is linked to increased cost to the newborn hearing screening system. Decreasing false-positive rates is required to prevent these unwanted effects.

One strategy that has been used to reduce false-positive rates is the use of two-stage screening tests (Mason & Herrmann, 1998; Clemens & Davis, 2001). Xu et al. (2011) reported a reduction in the false-positive rate from 4.9% to 2% as a result of changing from one-stage TEOAE screening to a two-stage TEOAE and AABR screening. Similarly, Roth et al., (2012)

found a 51% decrease in the false-positive rate using a two-stage screening protocol. Another strategy is the performance of repeated OAE tests when the newborn is older. Reductions of the false-positive rate have been shown when newborns were screened with OAEs at 10 days of age (Benito-Orejas et al., 2008; Tasci et al., 2010). It is also possible to reduce false-positive rates by performing the screening tests in a noise-controlled environment. Olusanya (2010) reported false-positive rates ranging from 1.4 to 13.8 % depending on the test environment. While these strategies have been shown to produce the desired effects, there are still logistic issues related to cost, follow-up attrition and time involved in screening.

Therefore the search for an ideal hearing screening protocol continues, as ongoing research uncovers new findings to improve both the OAE and AABR tests. The potential role of high-frequency OAE screening in reducing OAE false-positive rates has yet to be investigated. Thus, in this dissertation, it is proposed that OAE screening at high frequencies may be an efficient and affordable way to reduce false-positive results based on the facts presented above concerning specific frequency effects in newborns who constitute the false-positive group.

Use of animal models

Animal models have advanced the understanding of hearing function, and many novel techniques and approaches have begun with animal experiments. For two of the studies presented in this dissertation, the chinchilla was used to model the effect of amniotic fluid on OAEs at low and high frequencies. The chinchilla is a frequently used species for auditory experiments. It possesses an average minimum hearing threshold of 30 to 40 dB SPL when assessed behaviourally (Henderson, 1969; Miller, 1970) and objectively with auditory evoked potentials (Rothenberg & Davis, 1967; Miller 1970; Henderson et al., 1973). Furthermore, the estimated frequency range for hearing in chinchillas is 52–33,000 Hz, which is close to that of humans

(20–20,000 Hz) (Heffner & Heffner, 1991). The chinchilla also has multi-frequency tympanometry characteristics that are similar to those typically found in humans (Margolis et al., 1995). This facilitates comparison of results.

In addition, the chinchilla ear has a middle-ear space that is large enough to allow for the surgical manipulations that are frequently required in experimental designs (Vrettakos et al., 1988).

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CHAPTER 3 Otoacoustic emissions in newborn hearing screening: a systematic review of the effects of different protocols on test outcomes

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Preface

This paper is based on the results of a systematic review which focused on highlighting the influences of different screening protocols on OAE screening tests in newborns.

Abstract

Background and Objectives: Otoacoustic emission (OAE) tests are currently used to screen newborns for congenital hearing loss in many Universal Newborn Hearing Screening programs. However, there are concerns about high referral and false-positive rates. Various protocols have been used to address this problem. The main objective of this review is to determine the effects of different screening protocols on the referral rates and positive predictive values (PPV) of the OAE newborn screening test.

Methods: Eligible studies published in English from January 1990 until August 2012 were identified through searches of MEDLINE, Medline In-Process, Embase, PubMed (NCBI), ISI Web of Science, and the Cochrane Central Register of clinical controlled trials. Two reviewers independently screened the data sources, using pre-defined inclusion criteria to generate a list of eligible articles. Data extracted included the number of newborns screened, age at screening, OAE pass criteria, frequencies screened, number of retests, referral rates, and the number of newborns identified with permanent congenital hearing loss.

Results: Ten articles met the inclusion criteria, with a total of 119,714 newborn participants. The pooled referral rate was 5.5%. Individual referral rates ranged from 1.3% to 39%; the PPV from 2 to 40%. Increasing the age at initial screening and performing retests reduced the referral rate. Likewise, screenings involving higher frequencies had lower referral rates.

Conclusion: Delaying newborn hearing screening improves test results but may not be practical in all contexts. The use of higher frequencies and more sophisticated OAE devices may be useful approaches to ensure better performance of the OAE test in newborn hearing screening.

Keywords: Otoacoustic emissions, newborn, hearing screening, referral rates, positive predictive value, false positives, Review

Introduction

The goal of Universal Newborn Hearing Screening (UNHS) is early detection of hearing loss and prompt intervention through an integrated, interdisciplinary and family-centred approach[1]. UNHS seeks to screen all infants by one month of age, to conclusively diagnose permanent congenital hearing loss (PCHL) by the third month of life, and to treat the patient by the sixth month[1]. The current prevalence of PCHL in non-high-risk newborns is approximately 1 in 1000 for profound bilateral hearing loss, and 3–4 per 1000 for unilateral or mild cases[2-5]. Currently, otoacoustic emission (OAE) tests and/or auditory brainstem response (ABR) tests are employed as screening tools in newborn hearing screening programs (The screening ABR test is referred to as automated ABR (AABR), as opposed to a diagnostic ABR test). The OAE test is quick, easy to perform, and affordable, making it a good tool for newborn hearing screening programs[6]. It has therefore been used as an initial hearing screening method in many programs.

Previous reviews have shown that the benefits of UNHS outweigh the drawbacks and costs that are associated with the program[7]. Early diagnosis saves on the costs of intensive speech and language intervention and special educational services[8-12]. More children who underwent UNHS had their hearing loss diagnosed by the age of 9 months when compared with those who did not (67% versus 27%) [13].

Various sensitivity and specificity rates have been reported for the OAE test as a tool for screening newborn hearing. A sensitivity rate of 91% was recorded for newborns with mild PCHL[14]. Similar rates (91.7 and 92%) were shown by other studies using a two-step approach with OAE first and then AABR for those who failed[15, 16]. Up to 100% sensitivity values were reported by others[17, 18]. However, inadequate sample sizes have been a major limitation of these studies[19]. In addition, the use of AABR as the gold standard rather than the diagnostic (non-

automated) ABR test may partly account for heterogeneity in sensitivity and specificity reports, since AABR itself is a screening test that is subject to some of the challenges faced by the OAE screening test. An example is the systematic review by Wolff et al.[7] which used the AABR as the gold standard and found widely varying results (between 0.50 and 1.0 for sensitivity and between 0.49 and 0.97 for specificity).

In most reports on newborn hearing screening, sensitivity and specificity values cannot be provided because not all screening passes are followed by diagnostic ABR evaluation. Consequently, the outcomes of hearing screening programs have mostly been reported using the referral rate, the number diagnosed with PCHL and the number of false positives. OAE screening tests have been associated with high false-positive rates when used as the only screening test for newborn hearing[20-22]. False-positive referrals have been associated with transient conditions in the external auditory canal (e.g. collapse of the ear canal and the presence of debris) and middle ear (e.g. presence of amniotic fluid and mesenchyme) [23-25], as well as high ambient noise levels[26, 27]. The occurrence of false positives is a subject of concern because confidence in the screening program can be eroded and the follow-up diagnostic systems can be overburdened with unnecessary referrals [8, 28]. False-positive results also lead to avertable parental anxiety[21, 29-31].

Reducing referral rates and making them a better reflection of the true number of cases of PCHL has therefore been the focus of many newborn-hearing screening centres. The JCIH recommends that each screening program should adopt protocols based on what is practicable and suitable for its practice[1]. These protocols have varied with respect to the age of the newborn at first screening and the use of multiple tests, either with repeat OAE tests or with the AABR test performed on those who fail the initial OAE test. In addition to this, different OAE machines are in use, which in turn influences what frequencies are screened and what signal-to-noise ratio

(SNR) pass criteria are employed. The pass criteria of the OAE test are normally based on a preset SNR, with newborns being said to have passed the OAE test when they attain this SNR at a given number of frequencies. It is likely that these variations in protocols will affect the referral rates.

The challenge that faces UNHS today is that of reducing the referral rates while making use of a quick and cost-effective method of newborn hearing screening. This systematic review is aimed at evaluating the effects of specific OAE screening protocols. Therefore, only newborn hearing screening with OAEs alone are reviewed and discussed; studies that used the AABR test as part of the screening protocol were not considered. We examined the effects of age at screening, repeating OAE tests after a failed initial test, the frequencies tested and the SNR pass criteria on OAE test outcomes.

Materials and methods

Search strategy

A systematic review was conducted using the PRISMA guidelines[32]. A comprehensive search strategy was constructed to answer the research question. This search was run in five electronic databases: MEDLINE (OvidSP), Ovid MEDLINE In-Process Embase (OvidSP), PubMed (NCBI), ISI Web of Science, the Cochrane Central Register of Controlled Trials (CENTRAL) and other non-indexed citations. It was limited to articles published in English from January 1990 to August 2012. The search used medical subject headings, sub-headings, and free-text words including various words for newborns, hearing screening, otoacoustic emissions and auditory brainstem response. The complete search strategy for MEDLINE is shown in Appendix 1 (the search strategies for other databases can be obtained from the authors). The

titles and abstracts of the references of the articles selected for inclusion in the study, and of related reviews, were also searched to identify other eligible studies.

Inclusion and exclusion criteria

Newborn hearing screening studies on well babies that reported the use of OAEs as the initial screening test(s) and that included diagnostic ABR tests for newborns that failed the OAE test were included in this study. Articles had to include healthy newborns who underwent hearing screening with OAEs, either transient evoked (TEOAEs) or distortion product (DPOAEs). Studies that used a two-staged screening which involved an AABR test were excluded. For a study to be included, we required the number of newborns screened, the age at which they were screened, the number that passed or failed the screen, and the number that were eventually diagnosed with PCHL. Also required were the pass criteria of the OAE instrument used for screening as well as the frequencies that were screened, and a description of the retest protocol if there was one. We excluded case reports, conference abstracts, conference papers, presentations and reviews. Studies that pooled their results such that it was not possible to obtain clear data on OAE pass/fail rates or other required details, and those presenting data on mixed populations of high risk and healthy babies without presenting the data for the different populations separately, were also excluded.

Study selection

The first two authors independently screened the abstracts of references retrieved from the searches to obtain lists of relevant articles. The lists obtained after independent screening were then jointly reviewed to obtain a common list. This constituted the first-stage review. Where an abstract was not available and the title was suggestive of a newborn hearing screening program, the study was included for the second-stage review. Full texts of the relevant articles

were reviewed for the second-stage review to justify inclusion. Three authors were e-mailed for clarifications where important details were missing or unclear. In one of the included articles where the name and description of the OAE device was included in the manuscript but the pass criteria were not mentioned, and authors had not replied to our e-mail, we contacted the manufacturers of the OAE device. Disagreements between the first two authors were resolved by consensus.

Data were extracted from the eligible studies independently by the first two authors and were later jointly reviewed to ensure accuracy. The number of patients lost to follow-up was noted when provided.

We assessed the methodological quality and potential bias of included studies with a modified QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool[33] (Appendix 2). The risk of bias was rated to be low if the objectives of the studies were clearly stated, the main outcomes described and the spectrum of participants were representative of term newborns with low risk for hearing loss (all newborn population or random selection of newborns). The risk of bias was determined as low if diagnostic ABR was the reference standard, if all newborns who failed the OAE were offered a confirmatory test with diagnostic ABR by the third month of life as stipulated by JCIH. For the bias risk to be low, all newborns population or a random selection of newborns should have received the OAE test.

Data synthesis and statistical analysis

Our focus for data synthesis was on the following items: the total number of newborns screened, the number of newborns who failed the initial OAE screening test/retests, and the number with PCHL (i.e., who failed the diagnostic ABR). The secondary outcomes which were derived from the available data were: positive predictive values (PPV), referral rates, false

positive (FP) rates, and the prevalence of PCHL. The following formulae were used to calculate the secondary outcomes:

PPV = $N_{F\text{-}ABR} / N_{F\text{-}OAE}$

Referral rate = $N_{F\text{-}OAE} / N_{Tot}$

FP rate = $(N_{F\text{-}OAE} - N_{F\text{-}ABR}) / N_{Tot}$ (assuming $N_{Norm} \approx N_{Tot}$)

Prevalence = $N_{F\text{-}ABR} / N_{Tot}$ (assuming $N_{PCHL} \approx N_{F\text{-}ABR}$)

where

$N_{F\text{-}OAE}$ is the number who failed the OAE screening tests and was referred;

$N_{F\text{-}ABR}$ is thenumber who failed the diagnostic ABR test;

N_{PCHL} = number with PCHL;

N_{Norm} is the number without PCHL; and

N_{Tot} is the number screened.

The approximation $N_{Norm} \approx N_{Tot}$ is based on the fact that the expected fraction with hearing loss is very small. The approximation $N_{PCHL} \approx N_{F\text{-}ABR}$ is based on the assumption that the expected fractions of false-negative OAE and diagnostic ABR results are very small.

Sensitivity and specificity could not be calculated for any of the studies included in this review because none of the newborns that passed the screening tests was followed up with the diagnostic test.

Results

The search yielded 558 articles after the duplicates and the non-English articles were removed. Following independent and joint reviews of the titles, abstracts and full texts, only 10 fulfilled the inclusion criteria and were included in the review (Figure 3-1) [34-43]. The 10 included articles reported screening results on newborn populations from Taiwan (3), Sweden (1), France (1), India (1), Israel (1) China (1), Belgium (1) and the United Kingdom (1). Table 1 shows the characteristics of the studies included, and the various data extracted from the studies.

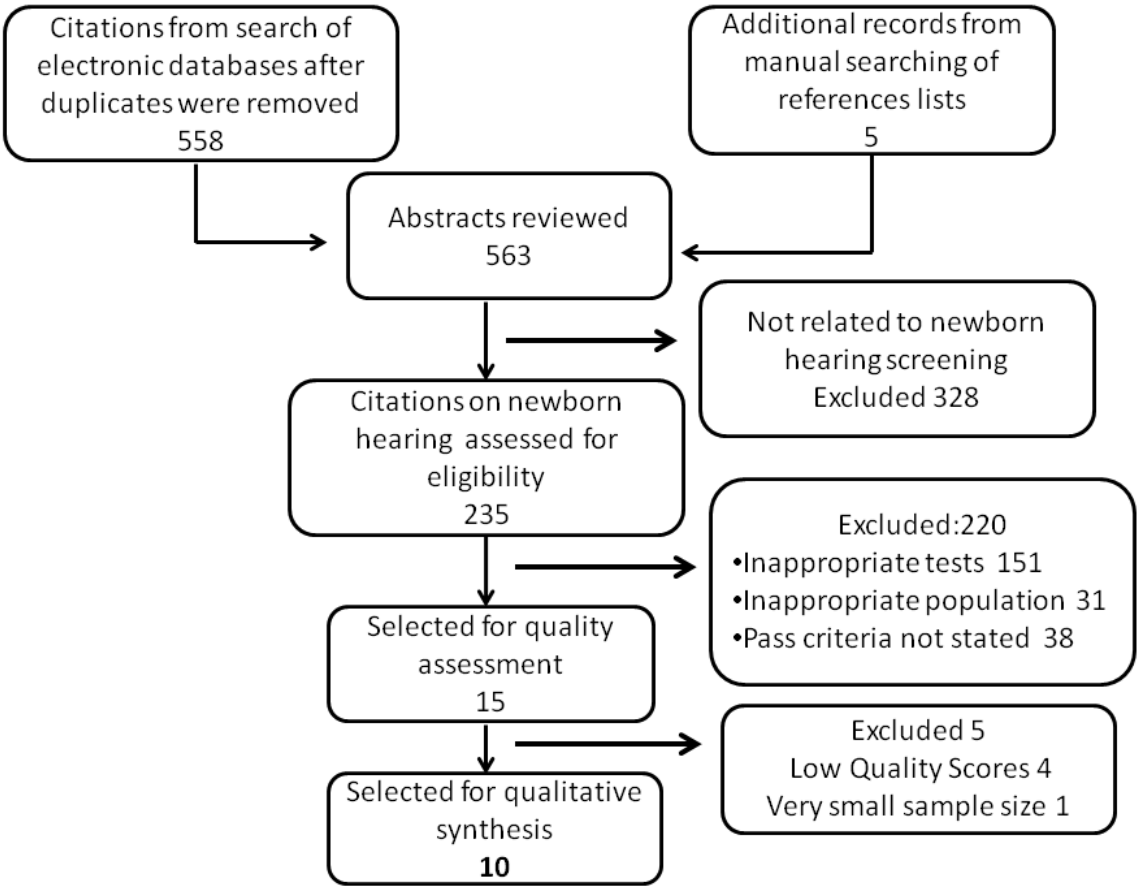

Figure 3-1: Flow diagram showing the process employed in selecting articles of interest

Most of the studies reported OAE screening tests at four frequencies, requiring a specific signal-to-noise ratio (SNR) at 3 or 4 frequencies for a pass. One study screened 5 frequencies, requiring 3 of these to have an acceptable SNR to pass. The frequency ranges tested were 1–4, 1.5–4, 1.4–4 and 1–6 kHz. SNR values reported were 3, 4, 5 or 6 dB above the noise floor. Some OAE machines had dual SNR pass criteria, requiring an SNR value of 3 dB in all four frequencies screened or 5 dB in 3 out of the 4 frequencies screened for a pass[34-36] (Table 3-1).

Author (Year)	**Study Location**	**Cohort size**	Age at screening (days)	**SNR pass criteria (dB)**	**Frequencies screened (kHz)**	**Referral rates/ (%)**	**False positive rate (%)**	**PPV**	**Prevalence of PCHL (%)**	**Lost to follow up**
Berninger & Westling (2011)	Sweden	31,092	3	3	1.5 - 4.0	9	8.8	0.02	0.18	NS
Wu et al. (2011)	Taiwan	1017	>2	6	2.0, 3.0, 4.0	3.7	2.95	0.21	0.79	0
Lin et al. (2007)	Taiwan	18260	>2	3 or 5	1-4	5.8	5.4	0.07	0.4	1.1
Leveque et al. (2007)	France	33,873	3	6	1.4-4	1.3	1.2	0.07	0.08	0.03
Mathur & Dhawan (2007)	India	1000*	≤2	3	1, 1.5, 2, 3, 4	21	19.5	0.07	1.5	0
Attias et al. (2006)	Jordan	7506	14-21	6	2, 3, 4, 5	2.5	2	0.2	0.51	3.2
Attias et al. (2006)	Israel	8089	>2	6	2, 3, 4, 5	0.48	0.3	0.4	0.19	0.04
Ng et al. (2004)	Hong Kong	1064	1-4	3 or 5	1-6	3.5	3.1	0.12	0.38	0.19

Lin et al. (2002)	Taiwan	6765	2.16	3 or 5	1-4	6.4	5.9	0.08	0.52	0.21
Govaerts et al. (2001)	Belgium	1772	3-5	6	1.6, 2.4, 3.2, 4	3.4	3.3	0.03	0.11	0.17
Watkin & Nanor (1997)	UK	9226	≤ 2	6	1.6, 2.4, 3.2, 4	13	12.4	0.05	0.6	11

Table 3-1 Characteristics of Included Studies (arranged according to year of publication).NS= Not specified

* Random selection

Two of the studies included newborns screened within the first 48 hours of life, while six strictly screened after 48 hours, one of them screening at 14–21 days of age[37](Jordanian population). One study[36] reported results of screening that was performed between 12 hours and 4 days after birth (Table 3-1).

The referral rates based on the first OAE tests were between 1.3% and 9% for 8 of the 10 included studies. Higher rates, of 13 and 21 % were reported by Watkin & Nador [38]and Mathur & Dhawan[39], respectively. The age at first test varied among the studies from 0.5 days to 21 days. The FP rates ranged from 1.2% to 19.5% and the PPV varied between 0.02 and 0.4. The wide ranges of values could be due to a number of factors, such as differences in the expertise of the operators, and the prevalence of hearing loss in the different populations. The measured prevalences of PCHL based on diagnostic ABR at 3 months (not including false negatives) were between 0.08 and 1.5%. The highest prevalence (1.5%) was reported for a randomly selected newborn population in India[39]. The referral rates were higher for the studies that had higher prevalences of PCHL.

The proportion lost to follow up (expressed as a percentage of the total number of newborns screened) also displayed a wide range, from 0% to 11%. Zero follow-up attrition was associated with relatively smaller cohort sizes [39, 43]. On the other hand, the highest attrition rate was reported for a district in the United Kingdom which ranked the 20th lowest of all local authorities in terms of overall deprivation[38].

Table 3-2 and Figure 3-2 show results from the six studies in which repeated OAE tests were done, highlighting a progressive reduction in referral rates with retests and specifically with increasing age at retests. Screening in the first day of life led to very high referral rates. Retests

are done at very different time points in the different reports: while some waited a few hours or days, others waited one week or two or more to repeat the OAE tests. Very low rates are reported when longer times are taken before retests are done; for example, Govaerts et al.[40] reported a 0.3% referral rate at 21 days, and similar improvements in referral rates were shown by Berninger et al.[41] and Marthur & Dhawan[39]. Very short retest times resulted in smaller but still noticeable improvements in referral rates[44].

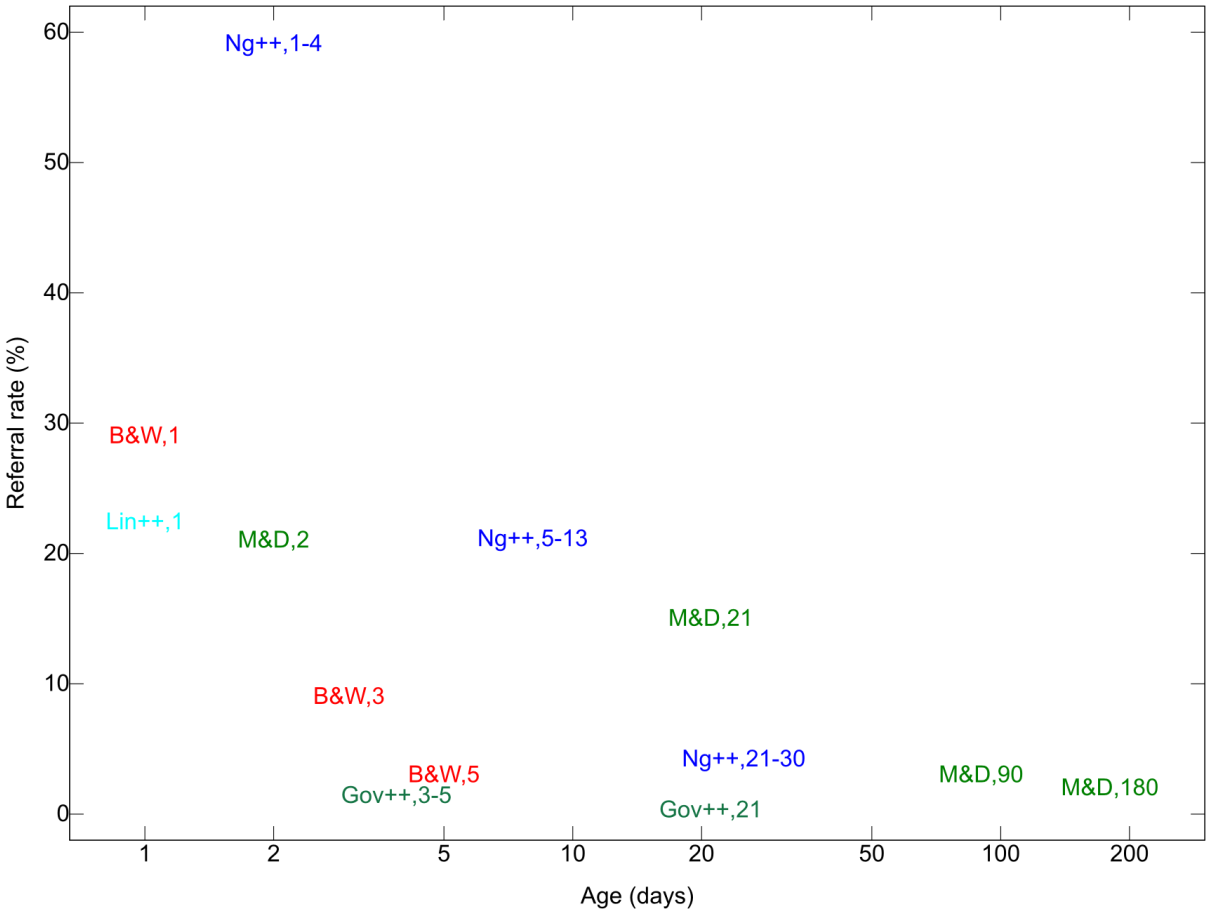

Figure 3-2: OAE referral rates at various newborn ages as presented in the included Studies. B& W Berninger & Westling (2011); M & D Mathur & Dhawan (2006); Ng ++ Ng et al. (2004); Lin ++ Lin et al. (2002); Govaerts ++ Govaerts at al. (2001); M & K McNellis & Klein (1997)

Source	Cohort size	Referral Rate (Age at screening in days)			
		OAE 1	OAE 2	OAE 3	OAE 4
Berninger & Westling (2011)	31,092	29% (1)	9% (3)	3% (5)	NA
Mathur & Dhawan (2006)	1000	21% (2)	15% (21)	3% (90)	2% (180)
Ng et al. (2004)	1064	59.1% (1-4)	21.1% (5-13)	4.2% (21-30)	NA
Lin et al. (2002)	6765	22.4% (2.16)	11.1% (*)	7.7% (*)	6.4% (*)
Govaerts at al. (2001)	1772	1.4% (3-5)	0.3% (21)	NA	NA
McNellis & Klein (1997)	50	39% (1)	24% (1)	15% (1)	2% (2)

Table 3-2 Decline in referral rates with repeat OAE tests. NA = Not applicable (study did not have further screenings) * Lin et al. (2002) did not provide timing of retests.

Data provided by individual studies were pooled in order to show the effects of the age at screening, the frequencies tested and the SNR pass criteria on the referral rates, PPV and FP rates (Table 3-3). The pooled referral rate when screening was done within 2 days of birth was 13.8%, while the rate was reduced to 4.7 % when screening was done after 2 days of life. The referral rate for a 3 dB SNR pass criterion was 9.4% while for 6 dB the referral rate was only 3.2%. The referral rate was higher for frequencies 1–4 kHz (6.1%) and 1–6 kHz (3.5%) than for 2–4 kHz and 2–5 kHz (1.6%). It is possible that the inclusion of 1 kHz amongst the frequencies screened is responsible for higher referral rates. This may also explain the higher referral rates with a 3 dB SNR pass criterion since all of the studies with this criterion also included 1 or 1.5 kHz among the frequencies screened.

		RR	PPV	FP rate
Age	**≤ 2 days**	1,409/10,226 (13.8%)	69/1,409 (0.05)	1340/10226 (13.1%)
	> 2 days	5,055/108,374 (4.7%)	254/5,055 (0.05)	4,801/108,374 (4.4%)
SNR	**3dB**	3,008/32,092 (9.4%)	71/3,008 (0.02)	2,937/32,092 (9.2%)
	6dB	1,964/61,483 (3.2%)	145/1,964 (0.07)	1,819/61,483 (2.96%)
	Dual*	1,524/26,089 (5.8%)	111/1,524 (0.07)	1,418/26,089 (5.4%)
Frequencies	**1-4 kHz**	6,199/101,988 (6.08%)	262/6,199 (0.04)	5,937/101,988 (5.8%)
	2-4; 2-5 kHz	265/16,612 (1.6%)	61/265 (0.23)	204/16,612 (1.23%)
	1-6kHz	37/1,064 (3.5%)	4/37 (0.11)	33/1,064 (3.1%)

Table 3-3 Pooled Data from Included Studies -showing the effects of age at screening, signal-to-noise ratio, and frequencies screened on the referral rate, positive predictive value and false-positive rate [RR = Referral rate, PPV = Positive predictive value, FP = False positive, SNR= Signal-to-noise ratio The numerator stands for the total number of newborns referred, while the denominator is the total number screened, pooled from all the included studies. * Dual SNR refers to OAE machines that require 3 or 5 dB SNR to pass at 4 or 3 frequencies respectively]

The pooled FP rates showed trends similar to those for referral rates. Higher FP rates were associated with screening within 2 days (13.2%), with screening using an SNR of 3dB (9.2%) and with screening involving 1–4 and 1–6 kHz (5.8% and 3.1% respectively). The pooled PPV was equivocal for the 2 age groups. However, the PPV was higher (0.07) for screenings using an SNR of 6dB or dual SNR than for an SNR of 3 (0.02), and the PPV was higher for screenings involving 2–4 and 2–5 kHz (0.23) than for screenings involving 1–4 and 1–6 kHz (0.04 and 0.11 respectively).

Discussion

Although UNHS has been widely accepted across the globe, it has continued to face challenges since its critical appraisal by Bess & Paradise[45] shortly after the first NIH Consensus Statement on the early identification of hearing impairment[46]. By convention, outcomes of newborn hearing screening programs are usually reported in terms of referral rates, which have also been used as a measure of the performance of screening protocols[28,47-49]. The JCIH guidelines stipulate that a good UNHS program should have referral rates of no more than 4%[1]. Given a prevalence of PCHL of approximately 1–3 per 1000 live births, a referral rate of 4% means that roughly ten newborns are referred for every actual case diagnosed. Hence, a 4% referral rate means that there are many false positives and efforts are required to reduce them further.

The results of this review show that referral rates were greater for programs that screened newborns within the first 48 hours of life (Tables 3-1 & 3-2). Conversely, referral rates were shown to be decreased with the use of repeat OAE tests (Table 2), supporting the idea that delaying screening reduces the number of newborns referred for full audiological work-up. This is consistent with the reasons previously given for false-positive outcomes with OAE testing: the

debris in the canal, and liquid and mesenchyme in the middle ear, resolve in the first few hours or days of life, making it likely that more newborns will pass the OAE test at later times. It is worth noting, however, that retests on the same day also showed a reduction in referral rates[44]. The fact that this happened with repeated tests when the babies' ears were presumably unchanged anatomically suggests that there is a significant random component in the results. It might also be that the initial tests resulted in removal of some debris from the ear, or that there were changes in the conditions in the newborn nursery, particularly in the levels of ambient noise.

Screening involving higher frequencies (e.g. 2–4 or 2–5 kHz) had lower referral rates than screening involving lower frequencies (1–4 kHz) (Table 3-3). The role of the middle ear in the transmission and detection of OAEs may explain this observation. Sounds (including OAEs) of different frequencies are transmitted differently through the middle ear[50,51] . The middle ear is made up of stiffness and mass components, like other vibrating systems. Conditions leading to increases in stiffness of the middle ear produce greater attenuation of lower-frequency sounds as they are transmitted through the middle ear, while changes in mass affect mostly the high frequencies[52]. The presence of amniotic fluid and mesenchyme in the middle ear, as may occur in early newborn life, reduces the effective volume of the middle-ear air space. This leads to an increase in stiffness and therefore affects the transmission of lower-frequency sounds. Lower screening frequencies might therefore be associated with greater referral rates. The mass component is also increased by entrained liquid[52]; however, studies investigating the effects of middle-ear liquid on OAEs at both high and low frequencies are needed to show how large the stiffness and mass effects are on OAE detection. Additionally, middle-ear anomalies have been shown to increase the noise floor in OAE measurements at lower frequencies[53, 54].

The SNR value in an OAE screening test represents the cut-off value acceptable for a pass. Increasing the SNR pass criterion would be expected to increase the referral rate, and in fact Watkin and Nanor[38] with a 6-dB SNR pass criterion did have a high referral rate. However, in the pooled data (Table 3-3) screening with a higher SNR criterion (6 dB) is associated with a lower referral rate. Factors related to technical differences amongst OAE devices may be the reason why high SNR values are associated with lower referral rates in our pooled data; there is continuous development in OAE technology with different algorithms being used in different OAE devices[55, 56].

Follow-up attrition rates are crucial when considering which protocol to adopt in a newborn hearing screening program. An 11% loss to follow-up was recorded by one of the studies included here[38] and even higher values (up to 65%) have been reported[57, 58]. Many factors have been associated with follow-up default in UNHS, including poor maternal socio-demographic characteristics, access problems (including lack of insurance), low maternal age, more than two other children, substance abuse, lack of prenatal education about newborn hearing screening, and lack of functional integration between hospital hearing information and Public Health[59]. Consequently, problems with follow-up make screening newborns at a later age problematical. If newborn hearing screening must be done in the nursery during the hospital birth admission, attention to the frequencies screened, the SNR criterion and the associated algorithms may help in reducing unnecessary referrals. It must be recognized, of course, that referral rates and false-positive rates must not be lowered without due attention to the costs of false-negative results.

There are a few limitations in this review. It was restricted to only publications in English; this could have eliminated studies in other languages with important results. The strict

selection criteria also meant that many potentially relevant studies were not included, in order to avoid data that could contribute biases. Since we do not have data on false negatives, the actual prevalences may be slightly higher than calculated. In addition, the fact that those who passed the OAE test did not receive further testing with ABR did not allow the evaluation of sensitivity and specificity profiles of the OAE test; therefore verification bias could not be ruled out. The occurrence of follow-up attrition is also a possible source of bias as participants lost to follow may differ from those who remain. Nevertheless, this review has some strength. A variety of population-based newborn hearing screening programs from different countries were included; the pooled data analyses also provided robust evidence based relationships between components of screening protocols and OAE test outcomes.

Conclusion

In this review we have focused on the performance of OAE screening protocols in curbing unnecessary referrals. Decreasing the false-positive rates of the OAE test will enhance its credibility and viability as an initial hearing screening test for newborns.

Influencing the age at screening, the frequencies screened and the SNR values accepted for a pass could be part of a strategy for ensuring a better performance of OAE tests. Although performing newborn hearing screening at an older age would lessen the occurrence of false positives, problems with follow-up are often said to make it impractical. However, combining UNHS screening with other routine newborn or infant health-facility visits after the hospital birth admission might alleviate this problem, at least in some health-delivery contexts. For example, Mathur and Dhawan[39] suggested that hearing screening could be conducted when newborns are brought to health facilities for immunizations at three months. Other time points that may be relevant include hospital visits for circumcision, and routine 6-week post-partum check-ups.

With this approach, however, there would be a need for more trained personnel available at those visits.

The use of more sophisticated OAE algorithms with high frequency resolution[60] and more robust artefact rejection and noise-floor estimation[61] may be useful approaches to improving DPOAE measurements. A deeper understanding of the contribution of middle-ear function to the different components of OAE test results may ultimately expand the utility of OAEs to include the provision of some information regarding the middle-ear status of the newborn and thus help to improve screening sensitivity and specificity.

Acknowledgements

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Appendix 1

Search Algorithm Medline (Ovid SP) 2012Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations 1946 to August Week 3 August 27, 2012

exp Neonatal Screening/ (6378)
(screening$ neonatal or newborn infant screening or screening$ newborn infant or infant newborn screening).tw. (21
1 or 2 (6391)
exp Mass Screening/ (92344)
(mass screening$ or screening$ mass or screen$).tw. (405071)
4 or 5 (437415)
exp Hearing Tests/ (35802)
(hearing test$ or test$ hearing).tw. (1053)
7 or 8 (36132)
exp Otoacoustic Emissions, Spontaneous/ or exp Acoustic Stimulation/ or exp acoustics/ or exp cochlea/ (78284)
((("oto-acoustic" or otoacoustic) adj3 emission$) or (("oto-acoustic" or otoacoustic) adj3 emit$) or spontaneous otoacoustic emission$).tw. (3810)
10 or 11 (78871)
exp Audiometry/ or exp Acoustic Impedance Tests/ (29073)
audiometr$.tw. (9580)
exp Evoked Potentials, Auditory, Brain Stem/ (6442)
((brain$ adj3 audi$) or (brain$ adj3 acoust$)).tw. (10319)
13 or 14 or 15 or 16 (41617)
3 or 6 or 9 (470973)
12 and 18 (9176)
17 and 18 (30596)
19 or 20 (32249)
limit 21 to (yr="1990 -Current" and "newborn infant (birth to 1 month)") (1247)
exp Random Allocation/ or exp Double-Blind Method/ or exp Clinical Trial/ or Meta-Analysis.pt. or Comparative Study.pt. or Review.pt. or random*.tw. or Meta-analys*.tw. or Meta analys*.tw. or metaanalys$.tw. (4077493)
((systematic* or quantitativ* or methodologic*) and (review* or overview* or synthes*)).tw. (107367)
23 or 24 (4116151)
22 and 25 (395)

Appendix 2

Modified QUADAS Tool

Population tested representative of general population
Acceptable standardized tool
Clearly described characteristics of the patients
Provision of estimates of random variability in the presented data
Descriptions of characteristics of patients lost to follow-up
Reference standard results blinded
Representative staff, places, and facilities
Representative test environment
Were un-interpretable results reported
Time between index test and reference standard
Clear definition of what was considered positive
Withdrawals
Partial verification avoided

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CHAPTER 4 Detection of otoacoustic emissions in chinchilla when the middle ear contains amniotic fluid

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Preface

In Chapter 3, it was shown that false-positive results are reduced if screening is done after 48 hours of birth, or if OAE retests are performed at a later date. This could be explained by the fact that the transient conditions of the middle and external ears become resolved and therefore there is less obstruction to the transmission of the OAEs through the middle and external ears. Amniotic fluid is frequently present in the middle ear of newborns as a consequent of swallowing activities in utero. This liquid travels via the Eustachian tube from the pharynx and may take up to two weeks post-natal to be cleared from the middle ear.

The effects of amniotic fluid on OAE detection has yet to be studied. Experimental studies have mostly focused on the study of normal saline as the middle-ear liquid. Other studies have created animal models of middle-ear effusion and then studied the effect of this on OAE detection. Also, clinical studies have been performed to determine OAE detection pattern in patients diagnosed with otitis media with effusion. The rest of this chapter focuses on the study of the effects of middle-ear liquid (amniotic fluid and normal saline) on otoacoustic emission detection.

Prior to the commencement of the study contained in this chapter, we determined the viscosity of 18 samples of human amniotic fluid. Fresh samples of human amniotic fluid were obtained from the birthing centre of the Royal Victoria Hospital, Montréal, Canada. The viscosities of these samples were measured using m-VROC micro-rheometer (Rheosense, San Ramon, CA). Shear sweeps were made to determine the relationship between shear strain and stress. Viscosity measurements were made for shear rates from 50 to 10000 s^{-1} and for temperatures at 37 °C.

Figure 4-7 in the Appendix shows the viscosity pattern of human amniotic fluid and normal saline as a function of shear rate. Amniotic fluid showed a non-Newtonian, shear-thinning behaviour like other biological fluids, unlike the Newtonian behaviour of normal saline: there was a sharp decline in the viscosity of amniotic fluid as the shear rate increased from 50 to 100 s^{-1}, and there was a further gradual decline in the viscosity up to a shear rate of 500 s^{-1}. Thereafter the viscosity remained constant at a value about twice that of saline, falling slightly at 10, 000 s^{-1}, where it becomes approximately the same value as that of saline, which was constant over the whole range of shear rates. The pattern was not affected by changes in temperature.

Abstract

Introduction/Objective: Otoacoustic emissions have frequently been used for newborn hearing screening. However, they have low specificities and high referral rates. The presence of amniotic fluid in the middle ear is one reason for these problems. The aim of this study was to determine the effects of human amniotic fluid on otoacoustic emissions and on the middle-ear function.

Methods: Forty-six chinchillas were randomly divided into 8 groups based on the type (amniotic fluid or normal saline) and volume (0.5, 1, 1.5, 2 ml) of liquid introduced into the middle ear. Distortion product otoacoustic-emission (DPOAE) and wideband energy-reflectance (WBR) measurements were taken under inhalational anaesthesia before and after introduction of middle-ear liquid. The differences in these measurements were subjected to statistical analyses.

Study Design: Prospective controlled animal study

Results: Significant reductions of DPOAE levels and increases in WBR occurred across all frequencies when there was liquid in the middle ear, and the changes became greater for

increased volumes of liquid. Changes in the noise level had important effects on the otoacoustic-emission signal-to-noise ratio at the three lowest frequencies.

Conclusion: Both human amniotic fluid and saline in the chinchilla middle ear resulted in changes in otoacoustic-emission detection patterns and wideband energy reflectance that may be relevant to newborn hearing screening.

Key words:

Otoacoustic emissions, middle ear, liquid, wideband energy reflectance, chinchilla, newborn hearing screening

Introduction

Many newborn hearing screening programs have found otoacoustic emission (OAE) tests to be good for initial screening because they are non-invasive, easy to perform and quick.[1] OAEs are generated within the cochlea, usually in response to external sound stimuli, and can be detected in the external auditory canal.[2] However, for OAEs to be detected in the ear canal they must traverse the middle ear (ME), so good ME function is essential for OAE detection.[3,4] Consequently, abnormal ME status is an important cause of absent or diminished OAEs in newborns undergoing hearing screening. Conversely, newborns that fail the OAE screening tests are more likely to have abnormal acoustic immittance test outcomes,[5] with 50% of newborn ears failing OAE tests reportedly having ME effusion or dysfunction.[6] Approximately four per thousand newborns had ME pathology with or without conductive hearing loss in one study.[7] The presence of amniotic fluid and mesenchyme in the newborn ME has been implicated as one of the reasons for abnormal ME function and consequent OAE test failures, particularly within the first few hours after birth.[8-11] Conditions with ME liquid (e.g., otitis media with effusion) impair sound transfer through the ME and result in absent or markedly reduced OAEs.[12-15] ME liquid increases the mass, stiffness, and resistance components of the ME, and therefore alters the sound-conducting function of the ME.[16] ME transmission of OAE signals is thereby affected, resulting in an OAE screening "referral" even when the cochlea is functioning normally. In the newborn the ME space is smaller than that of an adult,[17,18] so the presence of liquid produces even greater effects on ME function. Amniotic fluid in the newborn ME may persist several weeks after birth.[19] It is therefore likely that some newborns may still have some amniotic fluid in their ME at the time of screening, which is typically performed during the newborn's hospital birth admission.

The purpose of this study was to better understand the role of ME status in newborn hearing screening by investigating the effects of ME liquid on DPOAE detection at different frequencies and evaluating the relationships between DPOAE detection patterns and ME immitance. Although a number of authors have studied the effects of ME liquid (usually normal saline), this is the first report on the effects on OAEs of human amniotic fluid, which is what is actually found in newborns' MEs. The effects of normal saline were also studied in order to determine whether they are different from the effects of amniotic fluid. Newborn OAE hearing screening tests typically use frequencies ranging from 1 to 4 or 6 kHz. In this study we investigated frequencies up to 13 kHz, since higher frequencies might be less sensitive to the presence of ME liquid.[20]

Energy reflectance is the fraction of energy reflected backwards from the ME when sounds are introduced into the ear canal.[21,22] A value of 0 means that all of the sound energy is absorbed by the ME, while a value of 1 means that all of the energy is reflected back.[23] Wideband reflectance (WBR) measurements assess ME status over a wide range of frequencies, providing a more detailed analysis of the ME than conventional admittance measurements at 226 Hz or some other single frequency.[24, 25] WBR has been used in conjunction with newborn hearing screening to further classify ears that pass or fail the screening tests.[24, 26] WBR was used to evaluate ME status in this study.

A chinchilla model was used for this experiment because the superior wall of its ME space is thin and superficial, making it easy to inject liquid through the bony bullae into the ME.[27]

Materials and methods

The study received ethical approval of the institutional review board of the McGill University Health Center Research Institute. Animal experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Each of 46 adult female chinchillas (weighing between 400 and 600 g) was randomly assigned to one of eight groups, receiving either human amniotic fluid or normal saline in volumes of 0.5, 1.0, 1.5 or 2.0 ml in the ME. With 2.0 ml of liquid the chinchilla ME is essentially filled. There were at least five animals in each group. In each animal, one ear was randomly assigned to receive the liquid, with the other serving as control. Fresh samples of human amniotic fluid were obtained from the birthing centre of the Royal Victoria Hospital, Montréal, Canada. Only animals with good hearing (as determined by auditory brainstem responses) were included. We ensured normal ME status using WBR tympanometry (WB Tymp 3.2 model, Interacoustics, Denmark). All procedures were conducted under isoflurane inhalational anesthesia. OAE measurements were taken using a SmartOAE® DPOAE system (Intelligent Hearing Systems, Miami, FL).This DPOAE equipment is designed with animal experiments in mind and includes a special transducer for high-frequency DOAEs.

In order to avoid manipulations through the tympanic membrane, a trans-bulla route was used to introduce the liquid into the ME. A skin incision was made over the dorsal surface of the bulla and the bulla was punctured with a 21-gauge needle. Two punctures were made, one for introducing the liquid and the other to allow the escape of air and prevent pressure build-up in the ME. The liquid was introduced slowly to avoid perforation of the tympanic membrane and damage to other ME structures. The incision and punctured areas were then sealed using Vetbond^{TM} tissue adhesive (London, ON, Canada). Repeat measurements of DPOAEs and WBR

at ambient pressure were done with the head maintained in the prone position throughout the experiment. The animals were euthanized following the experiments with an overdose of ketamine and xylazine. In each animal, the ME was examined to confirm that there were no mucosal inflammatory changes.

DPOAEs ($2f_1-f_2$) were measured for f_2 frequencies between 825 and 13,250 Hz with two points per octave. Insert transducers were placed in the ear canal and paired primary tones were delivered at an f_2/f_1 ratio of 1.22 and intensities of L1 = 65 dB SPL and L2 = 55 dB SPL. We used chirp stimuli to measure WBR and equivalent volume at 60 frequencies from 0.24 to 8.0 kHz at ambient ear-canal pressure. We checked for an adequate probe seal in the ear canal by examining equivalent-volume values for outliers, large negative values (< -1.15 cm^3) at low frequencies being taken as indicating a leaky probe.[24] DPOAE and WBR measurements in ears with ME liquid were compared with those from control ears. For DPOAE measurements, the signal-to-noise ratio (SNR), which is a measurement of the level of DPOAEs in comparison with the level of background noise, was determined at baseline and compared with values obtained with different volumes of ME liquid (amniotic fluid and normal saline). The statistical significance of the changes in the DPOAE levels when the ME contains liquid was tested at each frequency using the paired *t* test. Differences were considered statistically significant when $p < 0.05$. In order to determine a pass/fail status at each frequency, we considered an SNR value of at least 5 dB as a pass, this being the criterion adopted for clinical applications by the OAE device being used. Logistic regressions were performed with the dependent variable being a pass or fail at each frequency; the predictor variables were the type of liquid, volume of liquid and test frequency.

Results

The baseline pattern of DPOAE levels in the chinchilla is presented as part of Fig. 1 for all 46 animals. There were no significant differences in baseline measures for the animals' right and left ears. The levels were small for the lower frequencies and increased progressively as the frequency increased, until a drop at the highest frequency. [*Mean OAE amplitudes for adult chinchillas at baseline are summarized in Figure 4-4, in the Appendix.*]

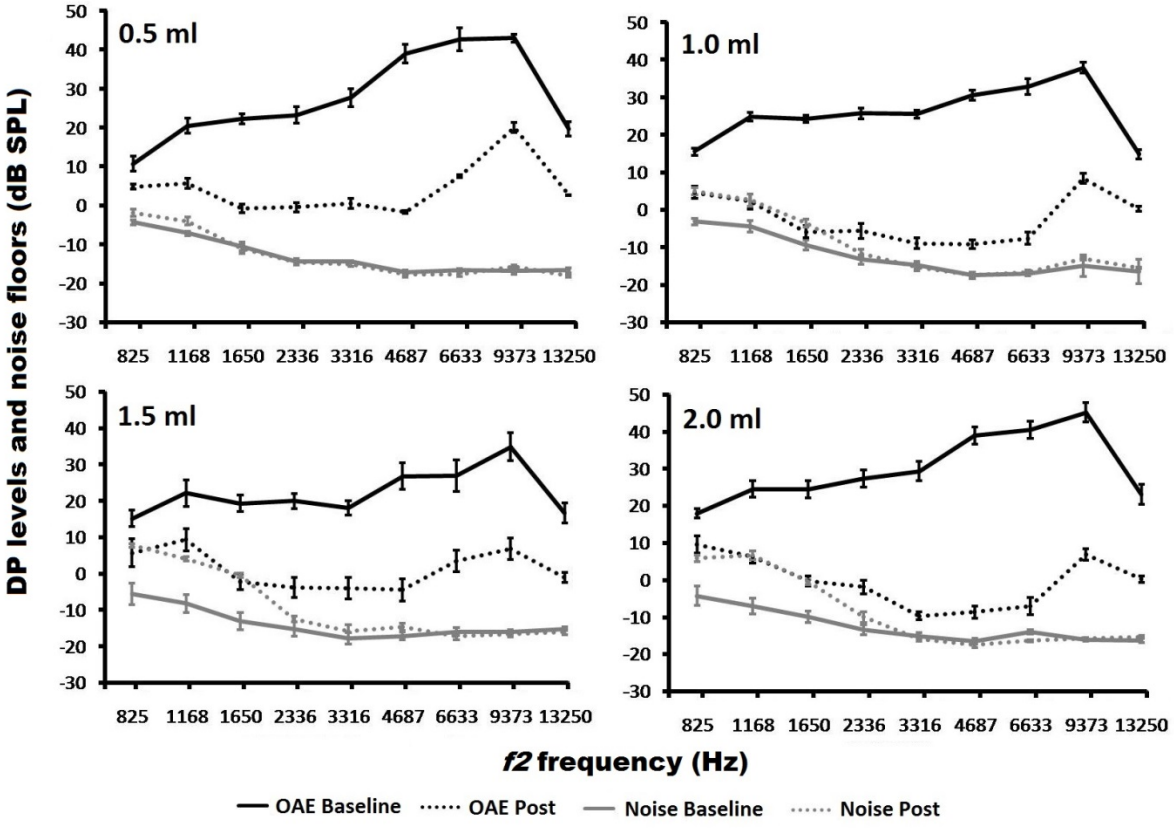

Figure 4-1 Mean DPOAE levels at baseline and with different volumes of amniotic fluid (AF) in the middle ear; error bars are SEM; n = 6 for 0.5, 1.0 and 2.0 ml groups, n = 5 for 1.5 ml group.

Figure 4-1 also shows a comparison of baseline DPOAE levels with those obtained following introduction of 0.5, 1.0, 1.5 and 2.0 ml of amniotic fluid. The OAE levels showed statistically significant reductions across all frequencies ($p \leq 0.0012$). The decrease in OAEs was greater for volumes above 0.5 ml. Similar results were seen with normal saline. The differences between the effects of the two liquids were not statistically significant for any of the four volumes.

Additionally, Fig. 4-1 shows how the noise levels of the OAE measurements were affected by liquid. There was a statistically significant increase in the noise floors with amniotic fluid at the lowest two frequencies for 0.5 ml and at the lowest three frequencies for 1.0, 1.5 and 2 ml ($p \leq 0.008$), while little or no change was observed in the noise floors at higher frequencies. Similarly, all volumes of normal saline in the ME gave rise to increases in noise floors at the lowest three frequencies which were statistically significant ($p \leq 0.006$) while at higher frequencies the changes in noise floors were negligible.

With ME liquid the SNR values were significantly decreased across all frequencies. Using a pre-set SNR pass criterion of 5 dB, as described above, the pass or fail status of each ear was determined. The ears that received 0.5 ml of either liquid obtained a pass at all frequencies, whereas the ears that received volumes greater than 0.5 ml had less than 5 dB SNR at f_2 = 825, 1168 and 1650 Hz and thus had a fail status at those frequencies. At higher frequencies, however, the SNR values with liquid volumes of 0.5 ml to 1.5 ml were greater than 5 dB and therefore still sufficient for a pass. With a volume of 2 ml, the SNR was greater than 5 dB only at f_2 = 9373 and 13250 Hz. Logistic regressions showed frequency and volume of liquid to be significant predictors of the outcome ($p < 0.0001$).

The SNR changes were similar for amniotic fluid and saline but a difference was observed at 1.0 ml. [*Figure 4-5, in the Appendix, shows the findings with normal saline in the chinchilla middle ear.*] While with 1.0 ml of normal saline the mean SNR was greater than the 5-dB cut-off (5.6 dB), with the same volume of amniotic fluid the mean SNR was below the cut-off (0.4 dB). However, logistic regression did not show the type of liquid to be a significant predictor of the outcome.

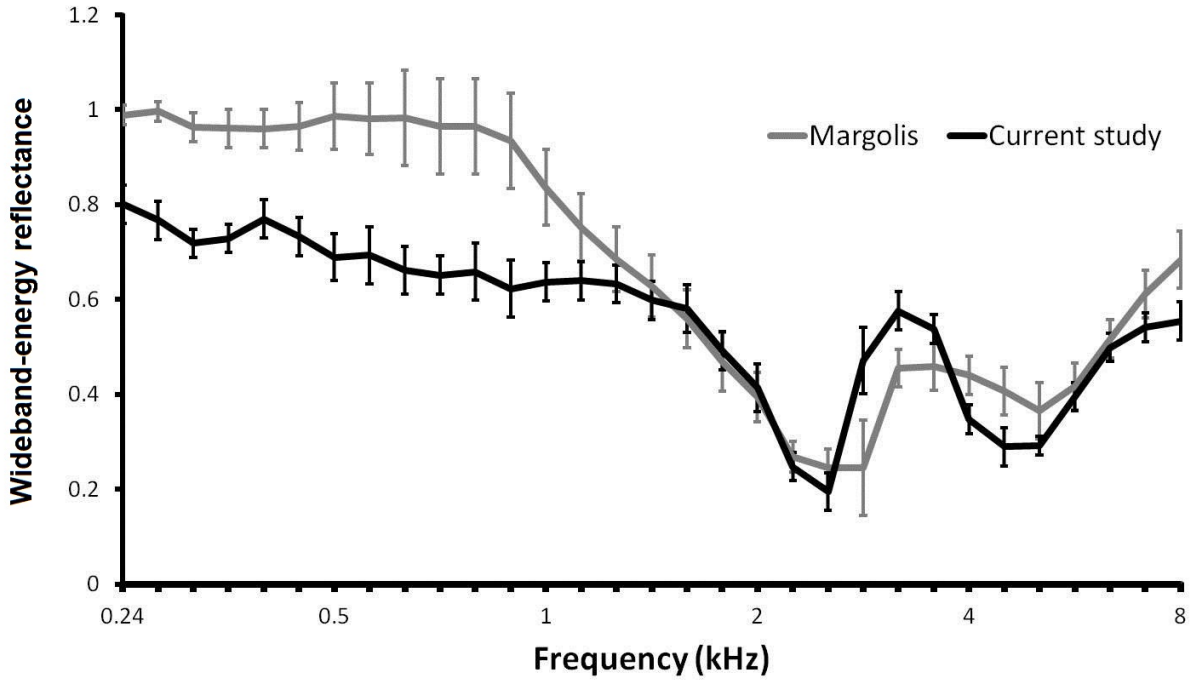

Figure 4-2 Baseline wideband reflectance (WBR) as a function of frequency; error bars are SD; $n = 46$.

Figure 4-2 shows the baseline WBR values at ambient ear-canal pressure for all 46 chinchillas. The chinchilla measurements of Margolis et al.[25] are included for comparison. As the frequency increases there is a gradual decline in the WBR until about 1.7 kHz, followed by 2

minima at about 2.4 and 4.8 kHz. With the introduction of either liquid, WBR increased progressively as the volume of liquid increased, across all frequencies. Figure 4-3 shows the effects on WBR with amniotic fluid. The WBR minima at 2.4 and 4.8 kHz were still present but were shallower in the presence of liquid. An increase in WBR of 0.45 for both liquids occurred at 2.4 kHz, while at 4.8 kHz the increases were 0.39 and 0.32 for amniotic fluid and saline respectively. At the maximum between the two minima the increase was only 0.12. The differences in the effects of amniotic fluid and of normal saline were not statistically significant. [*The findings with saline in the middle ear are shown in Figure 4-6, in the Appendix.*] Animals with smaller weights tended to have higher WBR values, but these differences were not statistically significant.

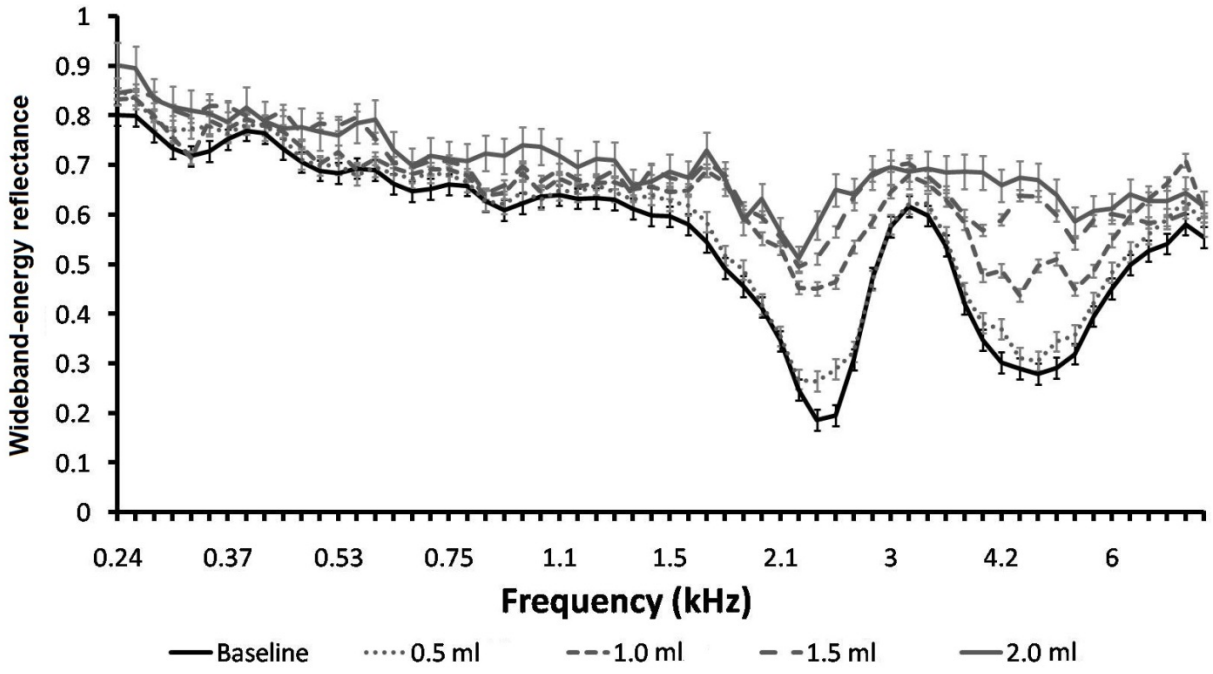

Figure 4-3 WBR at ambient ear-canal pressure as a function of frequency at baseline and with middle-ear amniotic fluid; error bars are SEM; n = 6 for 0.5, 1.0 and 2.0 ml groups, n = 5 for 1.5 ml group.

Discussion

We examined the effects of different volumes of the liquid in the ME on DPOAE test outcomes; our results show that the greater the volume of middle-ear liquid, the greater the effect on OAE levels. This is consistent with previously published data in which the middle-ear transfer function, as measured by the vibration of the tympanic membrane and the tip of the incus, decreased with increasing amounts of liquid injected into the ME.[28]

In our results, middle-ear liquid resulted in reduced DPOAE levels at all frequencies, presumably meaning that the middle-ear transfer function was affected for all the frequencies tested. Ueda et al.[29] reported similarly that DPOAEs at all frequencies were diminished when

normal saline was introduced into the bullae of guinea pigs. Some clinical studies have also shown reductions in DPOAEs at all frequencies with middle-ear liquid.[12]

A frequency-dependence of the effects of middle-ear liquid on either middle-ear function or OAEs has been found in some studies. For example, Ravicz et al.[30] found that reductions of umbo velocities in human temporal bones were different for high and low frequencies. In children with otitis media with effusion, greater reductions were shown at low frequencies.[13,15] In our study, although the effect of liquid on DPOAEs occurred at all frequencies, the noise floors at the low frequencies were elevated with middle-ear liquid so that greater reductions in SNR values were seen at the low frequencies.

OAEs depend on the ME for transfer of both the acoustic stimuli and the evoked emissions. Both mass and stiffness attributes of the ME may be altered when liquid is introduced. Stiffness is presumably influenced by the reduction in the effective volume of the middle-ear space, and perhaps other factors, while mass is presumably affected by the contact of liquid with the tympanic membrane and perhaps with other middle-ear structures.[30] It is therefore not surprising to find attenuation of OAE signals at all frequencies when there is liquid in the ME.

In this study, middle-ear liquid gave rise to decreases in DPOAE levels, increases in noise levels and decreases in SNR values; the changes in noise levels occurred mostly at the lower frequencies. The amount by which the OAE signals were above the noise floor became so small at low frequencies that they were undetectable (i.e., below the preset criterion of 5 dB). At baseline the OAE levels at low frequencies were already small, so that further reductions caused by middle-ear liquid resulted in undetectable OAE signals (Fig. 1). In a guinea-pig model, Ueda

et al. showed that DPOAE levels were indistinguishable from background noise with a liquid-filled middle-ear bulla.[29] This is at variance with our report of detectable OAEs at the highest two frequencies in chinchillas. In their study the bony bulla was opened to introduce the liquid, whereas we made use of needle punctures which were later sealed. In addition to the difference in methodology, there are differences between the guinea-pig and chinchilla ME which might account for the differences in the outcome at the high frequencies.[31,32] Noise-floor increases with middle-ear conditions have been reported previously.[33] We observe here that the increase occurs only at the low frequencies. Noise floors are known to be higher at low frequencies to start with[34] and conditions associated with increased ME impedance (e.g., the presence of ME liquid) may compound this problem.

At low frequencies the baseline WBR values were large (Fig. 2) and the OAE levels were low (Fig. 1). These observations are consistent, since high reflectance implies that less acoustical energy is passing into the ME, to ultimately get to the cochlea in order to evoke an emission. The occurrence of low-level OAEs at low frequencies is not peculiar to the chinchilla as other species[35] and also human neonates[36,37] have been shown to have lower levels at lower frequencies. High WBR at low frequencies has also been shown in human newborns and adults.[16]

In our study, middle-ear liquid gave rise to changes in the WBR across all frequencies, similar to what we showed for the OAE levels. WBR increased progressively with increasing volumes of liquid across all the frequencies (Fig. 3). This is consistent with the recent findings of Voss et al., who showed that WBR increased with increasing volume of liquid in the ME at most frequencies in cadaveric experiments and mathematical models.[38]

We found small differences in the effects seen with amniotic fluid and normal saline. These differences were not statistically significant except for the changes in the OAE SNRs for 1.0 ml of liquid. We suggest that viscosity may account for this, but the mechanism is not clear. Majima et al.[39] concluded that viscosity plays a role in the effect of liquid on middle-ear function, particularly below 1 kHz, but other studies have concluded that there is little or no effect of viscosity.[30, 40,41]

The chinchilla WBR curves presented here show similarities to those obtained by Margolis et al., who studied wideband reflectance in six chinchillas.[25] In both cases the reflectance pattern is characterized by minima at about 2.4 kHz and 4.8 kHz. However, Margolis et al. reported higher WBR (up to 0.98) at low frequencies than the values reported here (≤ 0.8). Although adult chinchillas were used in both studies, the differences in measurements could be due to the differences in the sizes of the animals used, middle-ear parameters having been shown to be dependent on body weight in other rodents.[42] A more recent clinical study also suggested variation in body sizes as a reason for differences in reflectance.[43]

Our results add to existing knowledge about the effects of middle-ear liquid (particularly amniotic fluid) on OAEs. These results suggest that it may be possible to reduce the number of false-positive screening results, and thus to increase the effectiveness of universal newborn hearing screening programs, by including middle-ear assessment with WBR and by including high frequencies in the OAE newborn hearing test. More research is needed to evaluate the usefulness and practicability of these suggestions.

Although the chinchilla shares some similarities with humans in terms of hearing, with DPOAEs being robust up to 10 kHz for both [44,45], the middle-ear volume of the newborn[46] is

much bigger than in the chinchilla. However, comparable effects of decreases in ME air volume on OAEs and ME function will be expected with liquid volumes that fill the same fraction of the newborn ME. Extrapolations from experimental animals to humans should of course be done cautiously.

Conclusion

In our chinchilla model, ME liquid produced significant reductions in DPOAE levels, increases in the WBR, and increases in the noise floors. Noise-floor changes occurred mainly at low frequencies, contributing substantially to the OAE outcomes.

Acknowledgements

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Appendix to Chapter 4

The following figures were not included in the manuscript corresponding to this chapter.

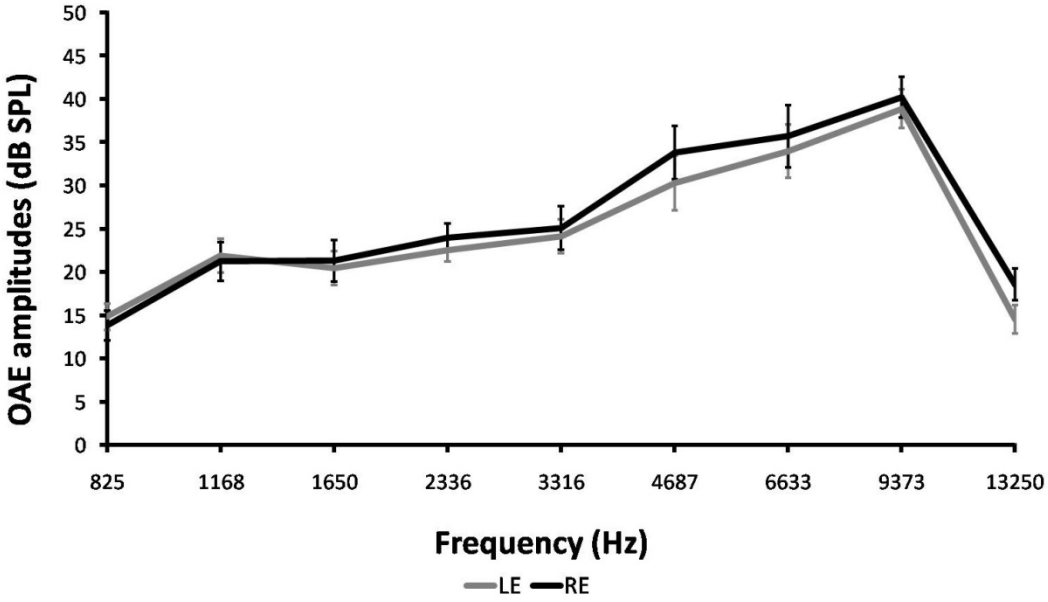

Figure 4-4 Mean OAE amplitudes in adult female chinchillas. Right and left ears without middle-ear liquid; n=46; error bars are SD.

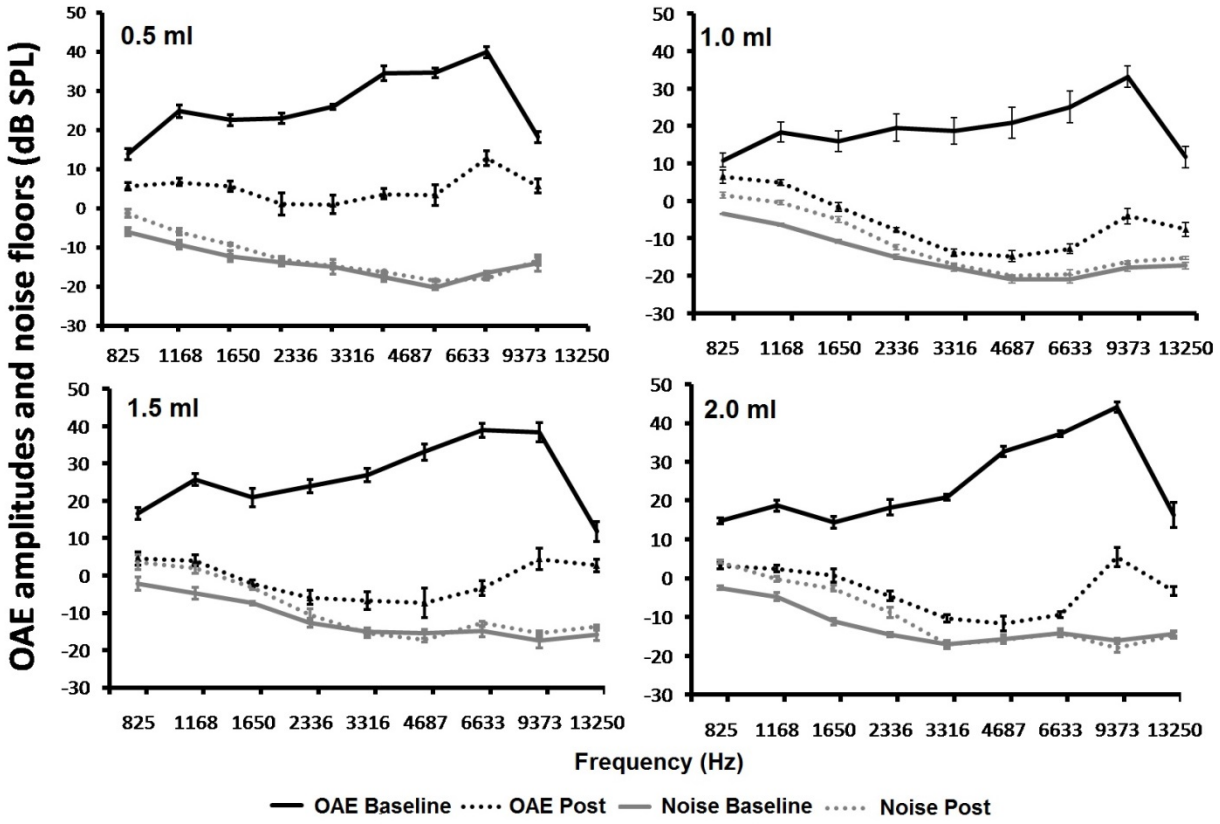

Figure 4-5 Mean DPOAE levels at baseline and with different volumes of amniotic fluid (AF) in the middle ear; error bars are SEM; n = 6 for 0.5, 1.0 and 2.0 ml groups, n = 5 for 1.5 ml group.

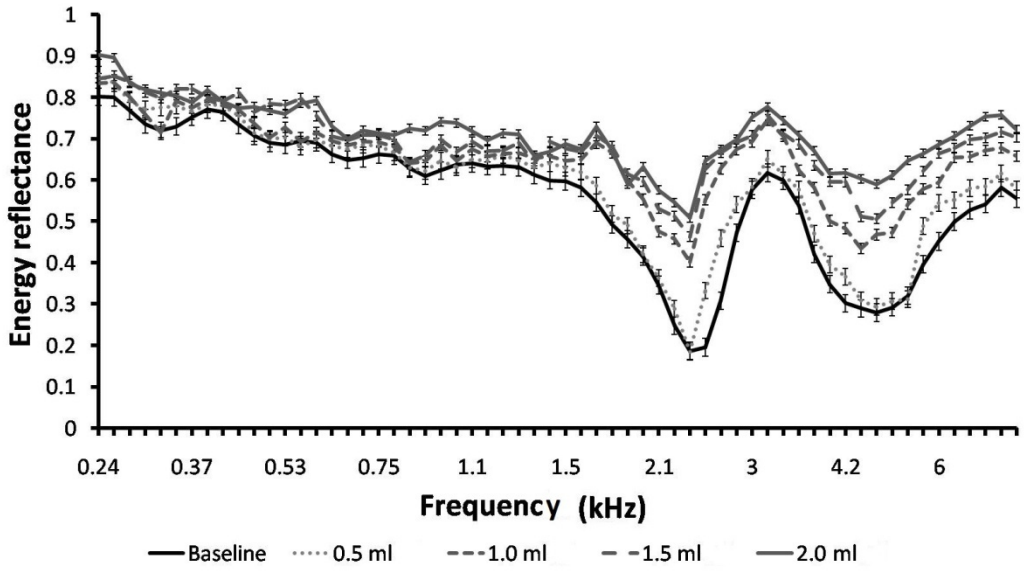

Figure 4-6 Energy reflectance at ambient ear-canal pressure (NS). Presented as a function of frequency at baseline and with middle-ear normal saline; error bars are SEM; n = 5 for 0.5 ml group, n=6 for 1.0, 1.5 and 2.0 ml groups.

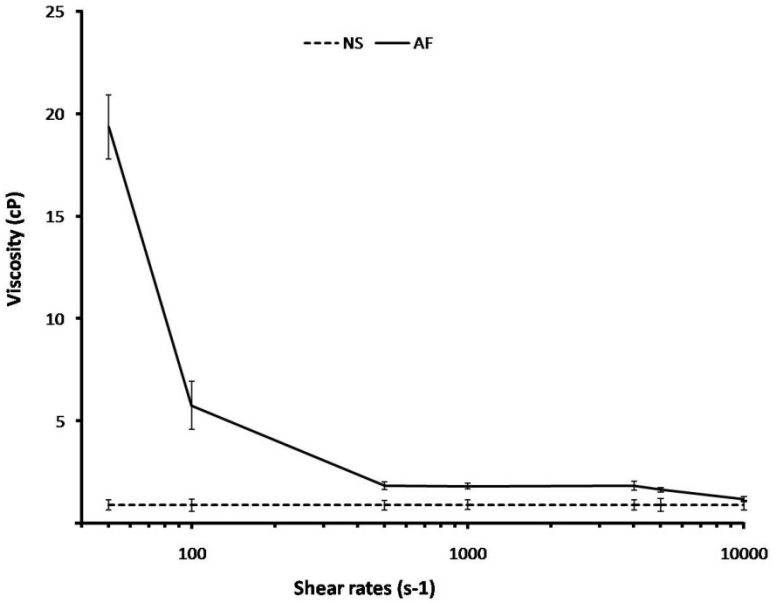

Figure 4-7: Viscosity of human amniotic fluid (AF) samples and of normal saline (NS) as functions of shear rate. Error bars are SD.

CHAPTER 5 Factors determining otoacoustic-emissions noise-floor levels

Olubunmi V. Akinpelu, W. Robert J. Funnell, Sam J. Daniel

Manuscript in preparation

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Preface

In Chapter 4 we showed that the presence of amniotic fluid in the middle ear resulted in significant increase in the noise floor at the three lowest frequencies. These led to significant changes in the signal-to-noise ratio which was an important determinant for pass or fail status at those frequencies. To our knowledge, there are two other studies reporting increase in noise floors as a result of middle-ear conditions. However, it is still not clear from what sources these noise-floor increases originate. In this chapter we show the possible sources of noise-floor changes when there is middle-ear liquid.

Abstract

Background: Noise floors in otoacoustic emission (OAE) tests originate from different sources and are important determinants of the signal-to-noise ratio, which in turn influences the test outcomes. Noise-floor increases have been reported in certain middle-ear conditions. It is, however, not clear whether the increase is from environmental or physiological sources or from the measuring equipment.

Objective: The objective of this study was to identify the sources of changes in noise floor in the presence of middle-ear liquid using two different experiments

Methods: [a] Animal Experiments: 20 adult female chinchillas (with normal hearing) were divided into 2 groups: live animals and euthanized (dead) animals; each received equal volumes of normal saline (1.0 or 2.0 ml) in their middle ears. Noise floors were measured with and without middle-ear liquid. [b] Cavity Experiments: Noise floors were measured in a 5-ml artificial cavity using two different OAE devices in two acoustically different environments (a double-wall acoustically insulated room and an uninsulated room). The probes were inserted in the lumen of the cavity in order to obtain cavity volumes varying between 1 and 5 ml.

Comparisons were made between the noise floors in the different conditions and differences were subjected to statistical analysis.

Results: Increases in the noise floor were observed at the low frequencies in both live and dead animals with middle-ear liquid. Similarly, the noise floor increased with decreasing cavity volumes, to a greater extent in the non-sound-treated environment.

Conclusion: Ambient noise and physiological sources are all contributory to noise-floor increases in certain middle-ear conditions.

Keywords Otoacoustic emissions, noise floor, low frequencies, admittance, ambient noise

Introduction

Otoacoustic emissions (OAEs) are produced by the outer hair cells of the cochlea; they are conducted through the middle ear and are recorded with a microphone in the external auditory canal. There are two basic categories of OAEs: spontaneous and evoked (e.g. Probst, 1990). The stimulus for evoked OAEs is usually one of two different types: clicks, which generate transient evoked OAEs (TEOAEs), and pairs of pure tones at frequencies f_1 and f_2, which generate distortion product OAEs (DPOAEs, e.g. Kemp 2002). Non-linear interactions between the two primary tones give rise to distortion products at frequencies including $2f_1–f_2$, $3f_1–2f_2$, $2f_2–f_1$ etc. (e.g. Goldstein, 1967; Kemp 1979). Typically, the $2f_1–f_2$ DPOAEs are measured in clinical protocols, since they are the largest distortion products in human ears in response to relatively low-level primary tones. The ratio of the intensity of the detected OAE response to the intensity of the noise is the signal-to-noise ratio (SNR), which is used in many contexts to determine the presence or absence of OAEs.

OAE signal levels in the external auditory canal are extremely low (10–20 dB SPL) and are often obscured by the noise in the external auditory canal (e.g. Hall & Chase, 1993). Therefore, the fundamental task in OAE measurement is that of the detection of signals within noise. In order to demonstrate the presence of OAE, its measured amplitude must be substantially greater than that of the noise (e.g. Whitehead et al., 1993). High levels of noise are therefore problematical in OAE tests. Particularly in newborn hearing screening, a pass or fail status for the OAE test is generally determined based on the attainment of a pre-set SNR criterion (e.g., Brass & Kemp, 1994). Changes in the noise floor will affect the SNR and can make the difference between a pass and a fail (e.g. Yang et al., 2002).

The noise floor in DPOAEs is often estimated by averaging the levels of several adjoining frequency components around the frequency of the distortion product of interest; this assumes that the noise level at the OAE frequency will be similar to those at the neighbouring frequencies (e.g. Avan & Bonfils, 1993).

Higher noise-floors have been shown to occur in the presence of high ambient noise in a hospital nursery (e.g. Jacobson & Jacobson, 1994). Certain middle-ear conditions have also been observed to be associated with higher levels of the noise floor. For example, in a study conducted by Owens et al., (1993) on OAEs among children with different middle-ear conditions , type B tympanograms (which are obtained either with middle-ear fluid or with tympanic-membrane perforations) were associated with higher noise floors for frequencies below 3 kHz as compared with type A tympanograms (which represent normal middle-ear function),

Similarly, Popelka et al. (1998) demonstrated higher OAE noise floors in newborns with middle-ear abnormalities. They suggested that the observed increases were due to increased acoustic impedance at the tympanic membrane, leading to the reflection of more of the noise energy present in the ear canal. However, with negative middle-ear pressures conditions, OAEs have been shown to have similar noise floors to normal middle-ear pressure conditions (Owens et al., 1993; Keppler et al., 2010).

The causes of the OAE noise-floor increases that occur in certain middle-ear conditions are still unclear. The aim of this study was therefore to investigate some of the reasons for such increases. We evaluated the effects of the environment, the OAE equipment and physiological noise on the magnitude and spectral characteristics of OAE noise floors using both animal and artificial-cavity models.

Methods

Animal experiments

Twenty adult female chinchillas (Moulton Chinchilla Ranch, Rochester MN) were used for the animal experiment, which was approved by the IRB of the Research Institute of the McGill University Health Centre and was conducted in accordance with the guidelines of the Canadian Council of Animal Care.

The chinchillas were divided into two groups of ten animals each. In group 1 the measurements were made in live, anaesthetized animals while in group 2 measurements were made in dead animals. The animals in each of the two groups were further subdivided into two groups with a different volume of liquid introduced into their middle ears (either 1.0 ml or 2.0 ml of normal saline). This experiment was designed to evaluate and compare noise-floor behaviour with and without the physiological input from the animal.

Each animal in both groups had its ears examined under inhalational anaesthesia to ascertain that the external auditory canals and tympanic membranes were normal. Only animals with normal middle-ear function as determined tympanometrically were included. With the animal in a supine position, baseline OAE noise-floor measurements were taken using a Smart DPOAE instrument (Intelligent Hearing Systems, FL). Subsequently, normal saline (either 1 or 2 ml) was slowly introduced into the middle ear via the transbullar route (Figure 5-1): two holes were made in the roof of the middle-ear bulla by puncturing with a 21-gauge needle, one hole for the injection and the other to allow release of air from the middle-ear air space as it was replaced by liquid, thus preventing a pressure build up in the middle ear. Repeat noise-floor measurements were then taken.

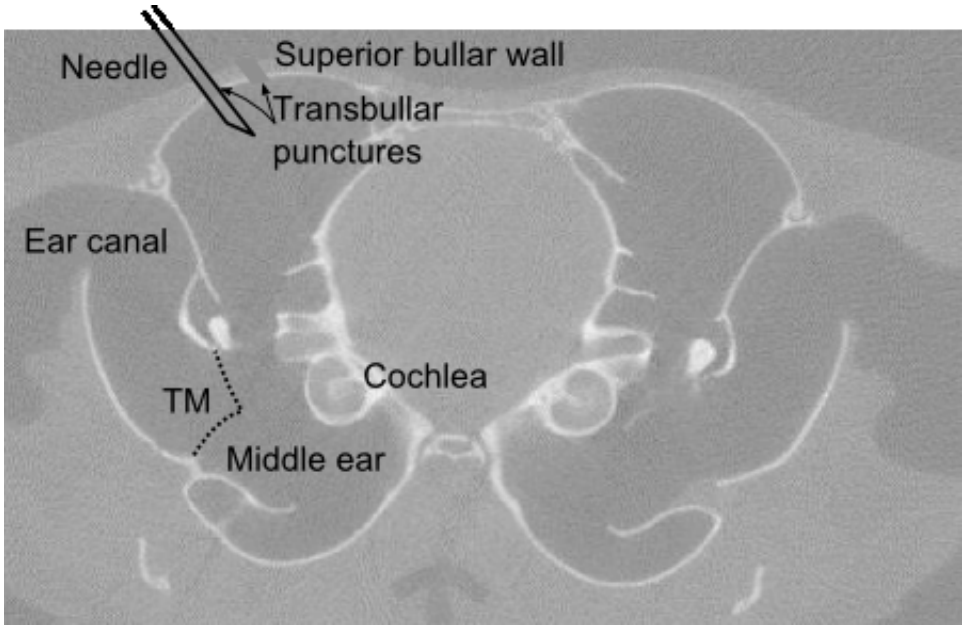

Figure 5-1: The transbullar route of injection in our chinchilla animal model.

Artificial-cavity experiments

OAE noise-floor measurements were also carried out with the OAE probe inserted into a 5-ml syringe at the 5, 4, 3, 2 and 1 ml marks, producing corresponding cavity volumes (Figure 5-2). The measurements were taken in a sound-treated room and in a non-sound-treated room. Measurements were taken with 2 different OAE devices: the Smart DPOAE (Intelligent Hearing Systems, Miami FL) and the ILO 292 USB-1 device (Otodynamics, Hatsfield, AL). For each cavity volume, measurements were taken twenty times spread over a 24-hour period; the averages and standard deviations obtained are presented in the *Results* section. The ambient noise levels of the sound-treated and non-sound-treated rooms were measured using a sound pressure level meter (System 824, Larson Davis, Denmark). These experiments were designed to examine the effects that ambient environmental noise and the use of different OAE devices have on noise-floor changes without the involvement of physiological factors.

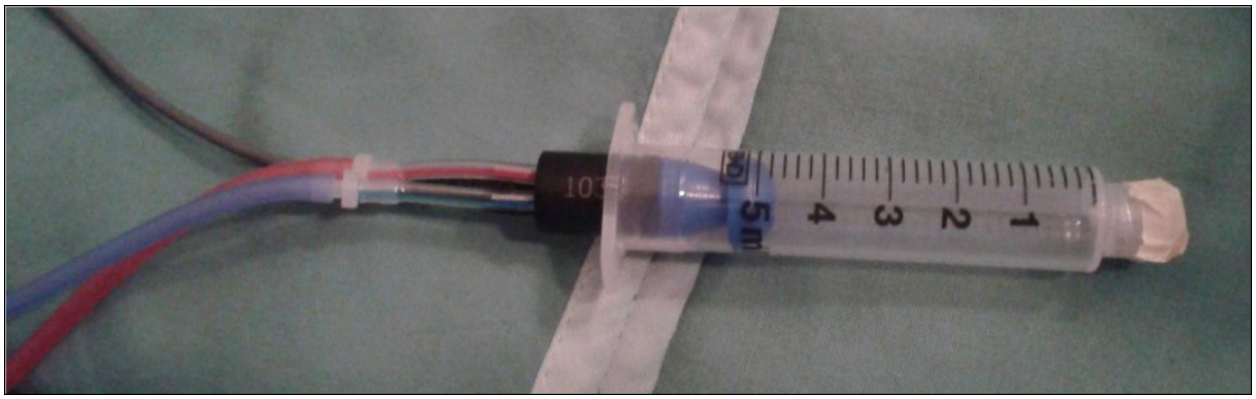

Figure 5-2: Artificial cavity consisting of a 5-ml syringe. The OAE probe is inserted into the lumen; here it is shown at the 5-ml position.

The ambient noise levels for both sound-treated and non-sound-treated rooms are shown in Figure 5-3. In both rooms noise levels were higher at low frequencies. For the non-sound-treated room, the measured ambient noise levels gradually declined from 53 dB to 47 dB up to 1000 Hz from where there is a steep decline to 15 dB at 8000 Hz. For the sound-treated room, the ambient noise levels were lower, but showed a similar decline from 34 dB to 8 dB up to 1000 Hz.

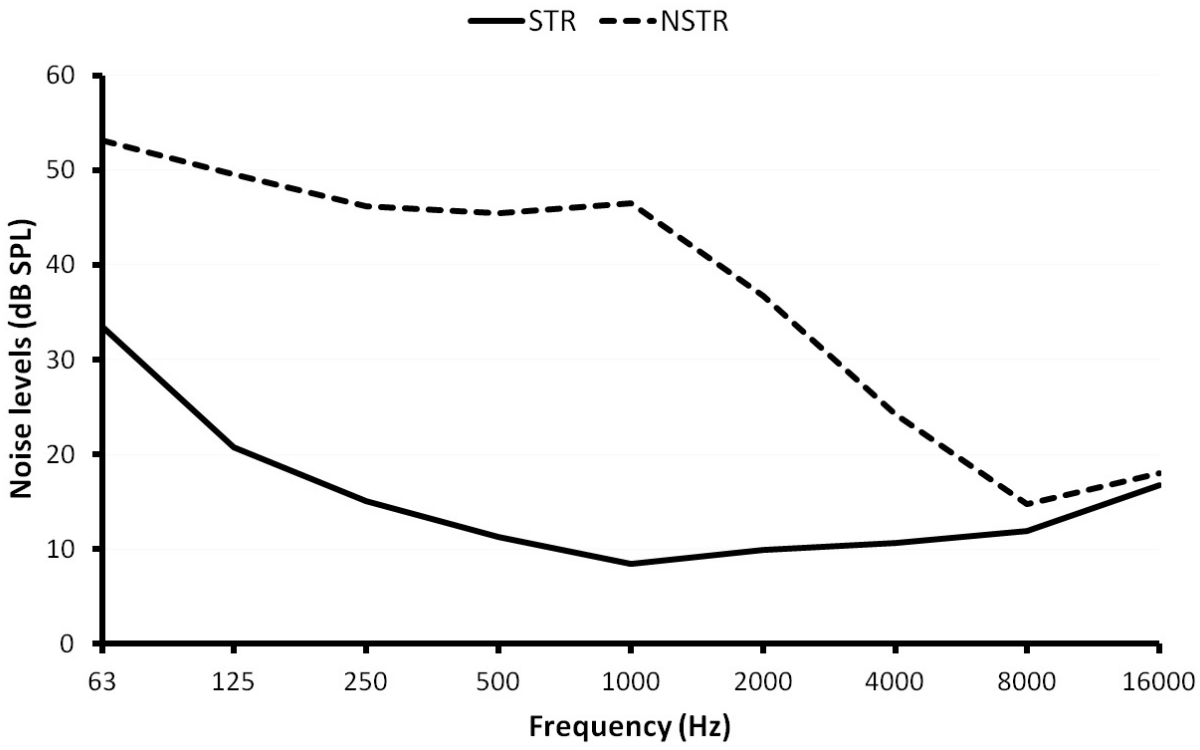

Figure 5-3: Ambient noise levels in the rooms where the measurements were taken. STR = sound-treated-room; NSTR = non-sound-treated room.

Data analyses

The data obtained from both experiments were analyzed using QuickCalcs (GraphPad, San Diego CA). The paired *t* test was used to compare mean noise-floor levels with and without middle-ear liquid; in live and dead animals; in sound-treated and non-sound-treated rooms; and with SmartOAE and ILO OAE devices. A p value < 0.05 was accepted as an indication of statistical significance.

Results

Animal experiments

Figure 5-4 shows the noise-floor measurements in both live and dead animals. The baseline noise floors were lower across all frequencies for the dead animals than for the live animals. (The baseline noise floors shown for the live-animal and dead-animal groups represent combined values for both the 1-ml and 2-ml volume sub-groups.) At the low frequencies, the noise floors at baselines were higher than those at the high frequencies. In the dead-animal group, the noise-floor levels at baseline dropped from -8 dB at the lowest frequency (825 Hz) to -20 dB at 3316 Hz, from where it became approximately constant up till 9373 Hz. On the other hand, in the live-animal group, the noise-floor levels dropped from -4 at the lowest frequency to -16 at 3316 Hz, remaining approximately constant till 13250 Hz At frequencies above 4000 Hz, the noise floors in the presence of middle-ear liquid were not significantly different from the noise floors without middle-ear liquid ($p \geq 0.37$). However, there was an increase in the noise floors at low frequencies in the presence of middle-ear liquid, especially at 825, 1160 and 1650 Hz, for both the live and dead animals ($p \leq 0.034$). Table 1 gives the details of the p values for the specific frequencies and animal groups. Approximately the same increases occurred with the 2-ml volume for both groups of animals: up to about 13 dB in the live-animal group and up to about 11 dB in the dead-animal group. The changes for the 1-ml volume were up to 9 for the live group but only about 6 for the dead group. The noise-floor increases in the live animals at the lowest frequencies were greater than the increases seen in the dead animals.

Frequency (Hz)	825	1168	1650	2336	3316	4687	6633	9373	13250
Live animals	0.0337	0.0282	0.0316	0.0413	0.4520	0.4291	0.4613	0.4501	0.3825
Dead	0.0178	0.0167	0.0214	0.0393	0.3741	0.3949	0.3958	0.5000	0.4030

Table 5-1: *p* values for comparison of noise floors with and without middle-ear liquid in live and euthanized animal groups

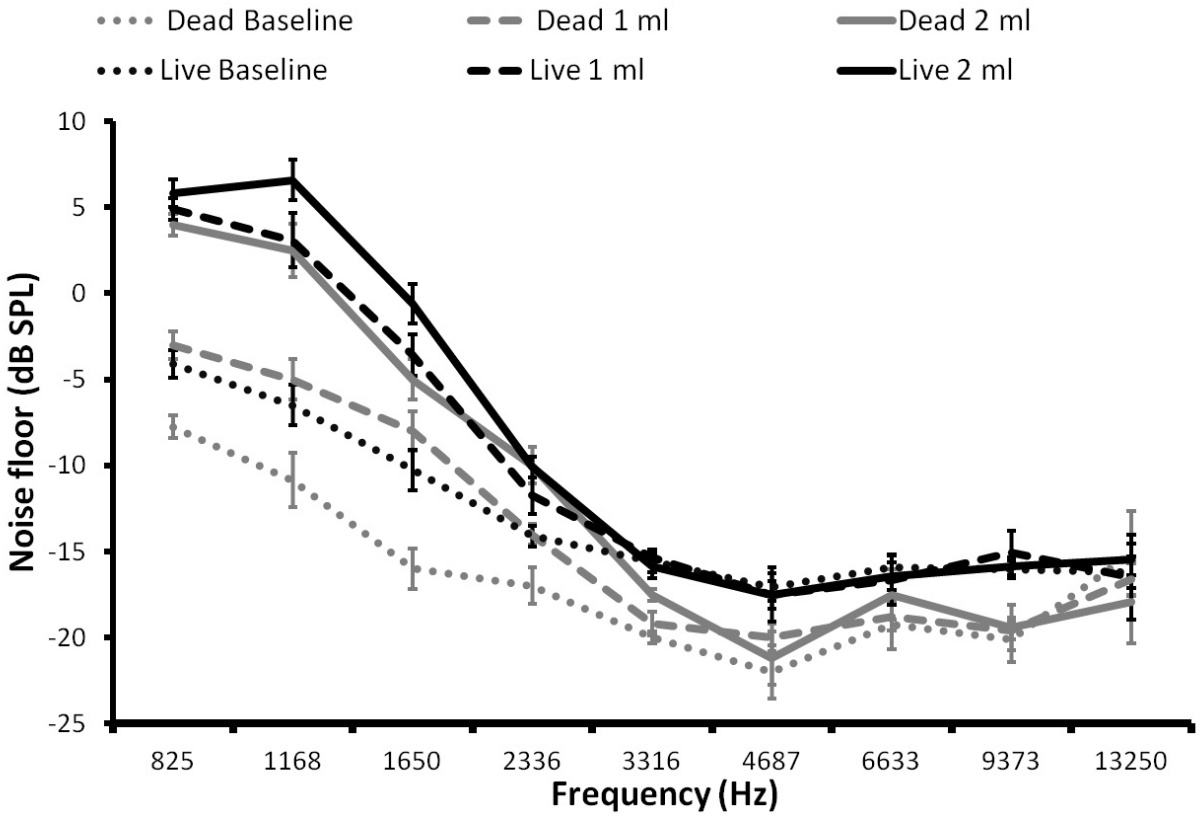

Figure 5-4: Noise-floor levels in live and dead chinchillas at baseline and with liquid. With 1.0 ml and 2 ml of normal saline in the middle ear. Grey lines are for noise floors in dead animals, black lines for live animals.

Artificial-cavity experiments

Figures 5-5 shows the noise-floor patterns recorded with the IHS OAE equipment in the artificial cavity in sound-treated and non-sound-treated rooms (STR and NSTR). Noise floors were higher at low frequencies in both rooms. In the non-sound-treated room, the noise floors increased with decreasing cavity volumes at frequencies below 2000 Hz; up to 24 dB increase was observed. On the other hand, the noise floors remained approximately constant, with decreasing cavity volumes; at frequencies above 4000 Hz. Similarly, in the non-sound-treated room, the noise floors increased with decreasing cavity volumes at frequencies below 2000 Hz. At frequencies above 4000 Hz, however, the noise floors were comparable for all cavity volumes.

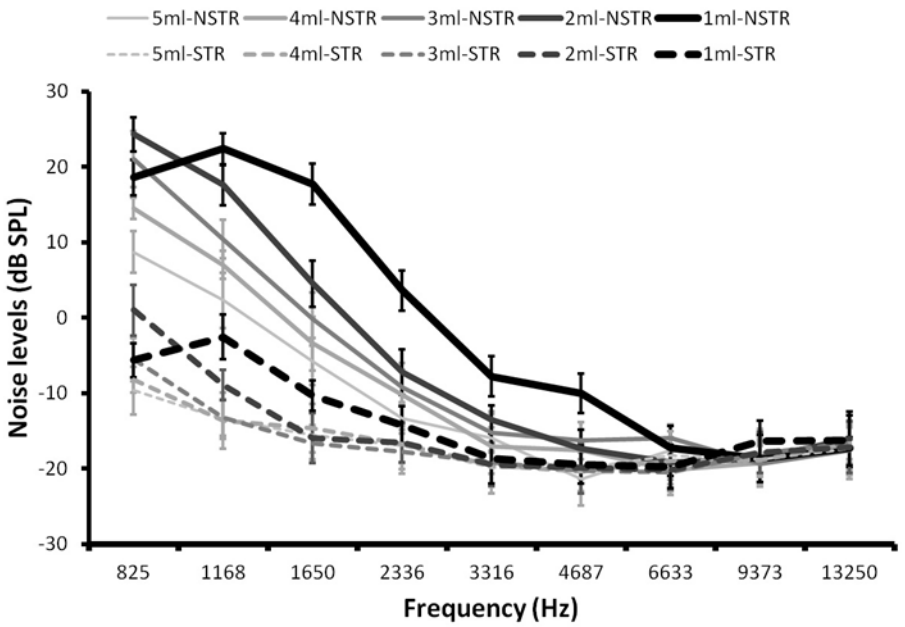

Figure 5-5: Noise-floor levels in an artificial cavity -SmartOAE (IHS Miami FL). Measurements for five different volumes and two different environments are shown. Broken lines are for measurements in the sound-treated room (STR), unbroken lines are for the non-sound-treated room (NSTR).

In the sound-treated room (Figure 5-5, broken lines), the noise floor declined from 825 to 1168 Hz, and then they flatten between 1650 through 6653 above which there was a slight rise. There were also increases with decreasing cavity volumes, however all noise-floor levels were lower in the sound-treated room, except at frequencies 6633 Hz, where they were comparable. The changes with decreasing cavity volumes were smaller in magnitude than those in the non-sound-treated room. For instance, at 825 Hz, a change of volume from 5 ml to 4 ml produced an average increase of 5.8 dB in the noise floor in the non-sound-treated room, but in the sound-treated room an increase of only 1.4 was seen.

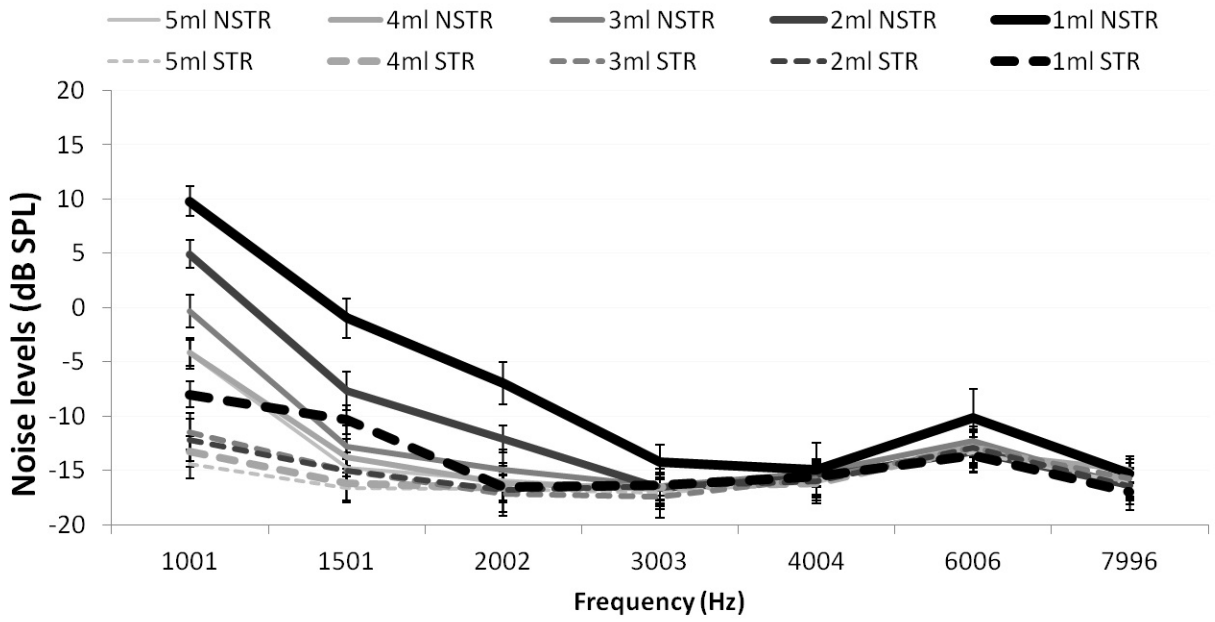

Figure 5-6: Noise-floor levels in an artificial cavity - ILO292 (Otodynamics, AL). STR=sound-treated room NSTR=non-sound-treated room.

Figure 5-6 shows the results of the noise-floor measurements in both sound-treated and non-sound-treated rooms using the ILO OAE equipment. The overall trends are the same as for the IHS equipment: The noise floors were generally higher at the low frequencies (although there

is a peak at 6006 Hz) and they decreased with increasing cavity volumes until they hit a lower limit. The noise floors were generally lower with the ILO device. At the lowest frequency, increase in the noise floor was sustained as the volume decreased from 2 ml to 1 ml, as opposed to what was observed with the IHS machine where 1 ml volume at the lowest frequency showed a decline in noise floor compared with the 2 ml volume.

Discussion

In this study we demonstrate frequency-specific increases in OAE noise floors in two different experiments. The animal experiment showed that increasing volumes of liquid in the chinchilla middle ear gave rise to higher noise floors at frequencies below 2000 Hz in both live and dead animals. In order to gain further insight into the source of the noise-floor changes, further experiments were performed in an artificial cavity. Changes in the volume of the cavity were used in this experiment to simulate admittance changes in a biological system. Decreasing cavity volumes produced noise-floor increases at the same frequencies as those seen in the animal experiment and with similar magnitudes.

The noise-floor increases seen in the dead animals with middle-ear liquid, and the similar increases seen in the artificial-cavity experiment, confirm that the source of the noise is not limited to the animal itself. However, although the noise floor was increased in the dead animal, the increase was not as large as that seen in the live animals; it is therefore reasonable to infer that endogenous physiological sources did contribute something to the increases in the noise floors seen in these experiments.

It is clear from the artificial-cavity experiments that the environmental component appears to be the greatest contributor to the noise-floor increase. A maximum increase in noise floor of 27 dB occurred as a result of the acoustic nature of the room where the measurement was

taken, whereas the maximum increase in noise floor as a result of volume change (5 ml to 1 ml) was 20 dB.

Similar noise-floor increases were observed with the two OAE devices. Different OAE devices use different methods for filtering, signal averaging and noise rejection. These differences will affect the outcome in terms of noise-floor levels. For example, longer averaging times have been shown to enhance DPOAE recordings at frequencies below 2 kHz (Stover et al., 1996; Lee & Kim, 1999). We also observed lower noise-floor levels with the ILO machine when compared with the IHS device however; similar noise-floor increases at the low frequencies, with decreasing cavity volumes were seen.

We have shown that the middle ear itself plays a role in the rise in noise-floor levels at low frequencies in the presence of middle-ear liquid. We suggest that a decrease in the effective middle-ear cavity volume (which occurs when there is liquid in it) may explain the increases in noise-floor levels. As in other vibratory systems, the middle ear contains both stiffness and mass effects, where stiffness effects dominate at the lowest frequencies and mass effects dominate at the highest frequencies (e.g., Osterhammel et al., 1993; Ravicz et al., 2004). A decrease in admittance (or an increase in stiffness) produces greater effects at the lower frequencies. Admittedly, middle-ear liquid will also increase the mass component with the potential effects at the high frequencies also. Our results have nonetheless not shown such effects on the noise-floor levels at the high-frequencies.

High levels of ambient noise have implications for OAE test outcomes (Hall & Chase, 1993; Jacobson & Jacobson, 1994; Hall & Muller 1997). The findings from this study also show that noise-floor increases seen at low frequencies in conditions associated with high impedance

are influenced by the ambient noise levels. It may be possible to reduce this unwanted increase in noise floors by making sure that ambient noise levels are within an acceptable range.

Our results have implications for the use of OAE as a screening method. First, OAE screening for newborns is mostly done in the nursery, which is not sound-treated. Second, transient middle-ear abnormalities are fairly common in the newborn population, particularly within the first few hours of birth, which coincides with the period of the birth admission, which is when newborns are typically screened for their hearing status. Third, the usual frequencies screened for are between 1 and 4 kHz. A closer look at noise-floor levels and their spectra in newborn OAE hearing screening results, particularly in those who have failed the test, might provide more insight into what role the noise floor plays in the outcome of newborn OAE hearing screening tests, particularly at the low frequencies.

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CHAPTER 6 High-frequency otoacoustic emissions in universal newborn hearing screening

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Preface

In Chapters 4 and 5 it was shown that the presence of middle-ear liquid led to increases in the noise floors at the low frequencies. These led to significant changes in the signal-to-noise ratio which was an important determinant for pass or fail status. The high frequencies were not so affected. Further investigations to determine if this phenomenon is repeatable in newborns was required with particular interest in determining if high-frequency OAEs would be a solution to the high occurrence of referrals and false-positive results associated with the newborn OAE hearing screening test. Since the inception of universal newborn hearing screening, little or no mention has been made on high-frequency OAEs in newborns. Hence, it was determined that a study looking at the pattern of high-frequency OAEs in newborns is needed. The newborns included in this study were those who were undergoing the conventional low-frequency OAE hearing screening. The study was designed to investigate the DPOAE amplitudes and noise floors in the group of newborn who passed and failed the OAE test. In particular we studied the high-frequency OAEs in the group of newborns who failed the conventional low-frequency OAE screening test but passed the automated auditory brainstem response test.

Although we were unable to ascertain the presence of middle-ear liquid in this group of newborns by conducting specific middle-ear function tests, our results suggest strongly that false-positive results occur because of peculiarities of the noise floor changes at the low frequencies.

Abstract

Introduction: Otoacoustic emissions (OAEs) are sounds of cochlear origin that are recordable in the external auditory canal. They are currently used in many newborn hearing screening programs as the initial hearing test, typically testing frequencies between 1 and 4 or 6 kHz, but

they have been associated with high false-positive rates. This study investigated the possible benefit of high-frequency (HF) OAEs for reducing false-positive rates.

Methods: Healthy newborns undergoing conventional hearing screening with an Eroscan distortion-product OAE device (MAICO, USA) and automated auditory brainstem response (AABR) were recruited for this study. High-frequency OAE tests were performed for f_2 frequencies 2, 4, 6, 8, 10 and 12 kHz using Otoread (Interacoustics, Denmark). The high-frequency OAE amplitudes, noise floors and signal-to-noise ratios (SNRs) for those who passed the regular screening test were compared with high-frequency OAEs of those who did not pass but passed the AABR test and of those who failed both the OAE and AABR tests. The effects of sex, side (left/right), mode of delivery, gestational age and birth weight on the outcomes of high-frequency OAE measurements were evaluated.

Results: 255 newborns participated in this study, including 138 males and 117 females. Of the 31 (12%) who failed the conventional OAE hearing screening, 23 (9%) passed the AABR test and 8 (3%) failed it. For an SNR threshold of 6 dB, OAEs tests at 4, 6, 8 and 10 kHz resulted in a reduction in the false-positive rate from 9% to 0.4%, or to zero if only three of the f_2 frequencies were required to exceed the threshold. Higher noise floors at 2 kHz contributed to the false-positive outcomes. SNR values were lower in newborns with birth weights greater than 4000 g, and they were lower at 2 kHz in newborns who were delivered at a gestational age of 41 weeks. SNR values were slightly higher in vaginally-delivered newborns than in those delivered by Caesarean section. At 2 kHz higher SNRs were seen with increasing age at screening in the group that failed the conventional OAE test but passed AABR.

Conclusion: HF OAEs were robust up to 10 kHz and resulted in a reduction in the OAE failure rate and the false-positive rate. These findings may be helpful in universal newborn hearing screening programs.

Key words: High frequency, otoacoustic emissions, newborn, hearing, screening, false positives

Introduction

Otoacoustic emission (OAE) testing assesses the function of the cochlear outer hair cells and has been used in many newborn hearing screening programs either as the sole test or as a preliminary test in combination with the automated auditory brainstem response (AABR) test. Newborn hearing screening with OAEs usually involves testing frequencies from 1 kHz to 4 or 6 kHz.

A major drawback of the OAE test, particularly in the newborn period, is the associated high false-positive rates (Clemens et al. 2000; Poulakis et al. 2003). In a recent review, OAE false-positive rates were shown to range from 1.2 to 19.5 % (Akinpelu et al. 2014). More newborns pass the test if repeated at later dates, but the logistics and cost implications of a return visit to the hospital make this problematic. Other methods that have been used to reduce these false-positive results include (i) repeating the OAE tests before discharge, and (ii) performing the AABR test in those who fail the initial OAE test.

Retained liquid in the middle ear in the newborn period may account for many false-positive results (Priner et al. 2003; Boone et al. 2005; Hunter et al. 2007; Boudewyns et al. 2011). (We use the word “liquid” rather than “fluid” in this paper in order to clearly distinguish it from air, which is also a fluid.) The presence of liquid in the middle ear reduces the middle ear volume and its compliance (e.g. Yimaz et al. 2008). A reduction in middle-ear compliance has greater effects on sound transmission at low frequencies than at high frequencies (e.g., Ravicz et al. 2004). Additionally, abnormal middle-ear conditions have been associated with increases in OAE noise floors (e.g., Owens et al. 1993; Popelka et al. 1998), and these increases have been shown to be greater at low frequencies (Akinpelu et al, under review). Therefore, high-frequency OAEs may be better detected than low-frequency OAEs in newborns with middle-ear liquid.

The usefulness of high-frequency OAEs in addressing the problem of false positives has not been investigated. Repeatable high-frequency distortion-product OAEs (up to 16 kHz) have been measured in human ears (Dreisbach & Siegel, 2001, 2005; Dreisbach et al. 2006), and OAEs up to 6 and 8 kHz have been used to monitor ototoxic reactions to drugs (e.g. Mulheran & Degg 1997; Stavroulaki et al. 2002), assess noise-induced hearing loss (e.g. Kuronen et al. 2003) and evaluate presbycusis (e.g. Lee et al. 2005).

The main aim of this study was to evaluate the usefulness of high-frequency OAEs in reducing false-positive outcomes associated with conventional OAE hearing screening tests. This involved examining OAE amplitudes and noise floors and the OAE pass/fail status at f_2 frequencies from 2 to 12 kHz for different categories of conventional OAE outcomes at the initial test. We also aimed to examine the effects on high-frequency OAEs of sex, gestational age, birth weight and the mode of delivery (vaginally or by Cesarean section).

Methods

Ethics approval was obtained from the Institutional Review Board of the McGill University Health Centre before commencing the study. Newborns were randomly selected for inclusion in the study, subject to logistical constraints. Prior to testing, parents of the newborns were given written and oral explanations of the study, questions were addressed and informed consents were obtained. The tests were conducted in the normal newborn nursery of the Royal Victoria Hospital (RVH), Montréal. All newborns included were term (from 37 to 41 completed weeks). Newborns were excluded if they had a family history of hearing loss or perinatal medical conditions that could pose a risk to hearing. We excluded newborns with neonatal infections being treated with gentamicin, those with hyperbilirubinemia and those with history suggestive of birth asphyxia.

Each baby was first tested in the well-baby nursery by an audiology technician with the conventional distortion-product OAE (DPOAE) screening device (EroScan, MAICO, U.S.A.) used by the RVH newborn hearing screening program. The probe consisted of two primary tones, f_1 and f_2, with f_1/f_2 = 1.22 and with the primary levels L1 and L2 being 65 and 55 respectively. The $2f_1$-f_2 distortion product (DP) was measured. The EroScan device uses a fixed-averaging-time approach as a stopping rule. This test (referred to below as the initial or conventional test) used OAEs at f_2 frequencies of 1.5, 2, 3, 4, 5 and 6 kHz. The criterion for passing was a signal-to-noise ratio (SNR) of 6 dB or more in at least 4 of the 6 f_2 frequencies tested. Newborns who fail this initial test are referred immediately for the second-stage screening with AABR using ABaer (Bio-Logic, IL). This machine uses a 100-microsecond click stimulus at 35 dB intensity. It automatically evaluates the response with a point-optimized variance-ratio (POVR) signal-detection algorithm. A pass or refer is recommended based on the comparison of the POVR with a preset criterion. The test is terminated when one of the following happens: a POVR score of 3.5 is achieved after a minimum of 1536 stimuli have been averaged; a total of 6144 stimuli are averaged and the POVR score reached a value of 3.1 or higher; or the POVR score does not reach a value of 3.1 at the end of two sets of 6144 stimuli.

In our study, the newborns (regardless of their pass or fail status at the initial test) were also tested with the OtoRead distortion-product OAE device (Interacoustics, Denmark) for f_2 frequencies of 2, 4, 6, 8, 10 and 12 kHz within an hour of the initial test. For this second OAE measurement (referred to below as the high-frequency test) the noise floor and OAE amplitudes were retrieved for analysis in addition to the SNR. In order to allow for comparison of our outcomes with those of the conventional OAE test, we accepted an SNR value of 6 dB above the noise floor as a pass criterion for each frequency we tested. The OtoRead device has the same

probe specifications, preset protocols, stimuli and stopping rules as the EroScan device. The EroScan OAE device was configured to give only a pass/fail result at the end of the hearing screening, so separate OAE and noise-floor data were not available. For the OtoRead device, the DP levels were less than or equal to the noise levels at all frequencies when measurements were taken with a 5-ml coupler.

Recordings were done with the OAE probe inserted as deeply as possible in the canal while making sure that the baby was not in discomfort. The complete DPOAE test of the OtoRead (and EroScan) instrument begins with the calibration phase in which responses from a sequence of calibration tones are measured; the voltages needed to obtain the desired sound pressures are calculated. A successful calibration leads to the actual test, which consists of measuring the response obtained from the pairs of test frequencies (f_1 and f_2). Frequency-domain estimates of the actual P1, P2, DP and noise floor are obtained using a Fast Fourier Transform. The noise floor is estimated by averaging the power in the four bins closest to the DP bin.

For the purposes of data analysis, the newborns were grouped into (i) pass or fail (with reference to the outcome of their initial hearing screening test), (ii) male or female, and (iii) delivery by Caesarean section or vaginally. They were also sub-divided based on their birth weights and gestational ages at birth. The data analyzed included OAE amplitudes, noise floors and SNRs.

The data were analyzed using two-way mixed analysis of variance (ANOVA) that independently examined the effects of several independent variables on the dependent variable of DPOAE SNR (measured in dB) over six f_2 frequencies (2, 4, 6, 8, 10 and 12 kHz). The independent variables included: (i) outcomes on the initial OAE and AABR tests (newborns who

failed the initial OAE test and also failed the AABR test, newborns who failed the initial OAE test but passed the AABR test, and newborns who passed the initial OAE); (ii) age at screening (12-24 hours, 25-36 hours and 37-48 hours); (iii) birth weight (less than 2499 g, 2500–2999 g, 3000–3499 g, 3500–3999 g, and 4000 g or more); (iv) gestational age (37, 38, 39, 40 and 41 weeks); (v) sex (male or female); and (vi) ear tested (left or right). Post-hoc tests were done using *t*-tests. Chi-square tests of independence were performed to examine the relationship between the pass/fail status on our high-frequency test (accepting an SNR value of 6 dB or more as a pass at each frequency) and the frequency tested; Fisher's exact test was used when a cell had fewer than 5 participants.

Results

Overview

There were 255 newborns included in the study, of whom 138 (54%) were males and 117 (46%) females. There were 160 (63%) delivered vaginally and 95 (37%) by Caesarean section. The initial OAE tests were done within 24 hours of birth in 84 cases (33%), after 24 hours but before discharge from the hospital in 171 (67%). The birth weights ranged between 2390 and 5290 grams, and all newborns were delivered at or after 37 completed weeks of gestation. The initial test was passed by 224 (88%) newborns; 23 (9%) failed the initial test but passed the AABR test, four cases being unilateral failures; and 8 (3%) failed both the initial OAE test and the AABR test, all of them bilaterally.

Figure 6-1 shows the high-frequency OAE SNRs for all newborns. The SNRs were robust for f_2 frequencies 2 to 10 kHz (Figure 6-1). There was a slight progressive increase in the mean SNR from about 16 dB at 2 kHz to 18 dB at 8 kHz followed by a sharp drop-off to about 13 dB at 10 kHz and only about 5 dB at 12 kHz. An SNR value of at least 6 dB was present at 12

kHz in 99 (39%) of all the newborns. Females had significantly greater SNRs than males on average ($F(1,253) = 5.03$, $p = 0.03$, $\eta^2 = 0.02$, Figure 6-1). There was no statistically significant difference between left and right ears ($F(1,490) = 0.35$, $p = 0.56$, $\eta^2 = 0.001$, Figure 6-2).

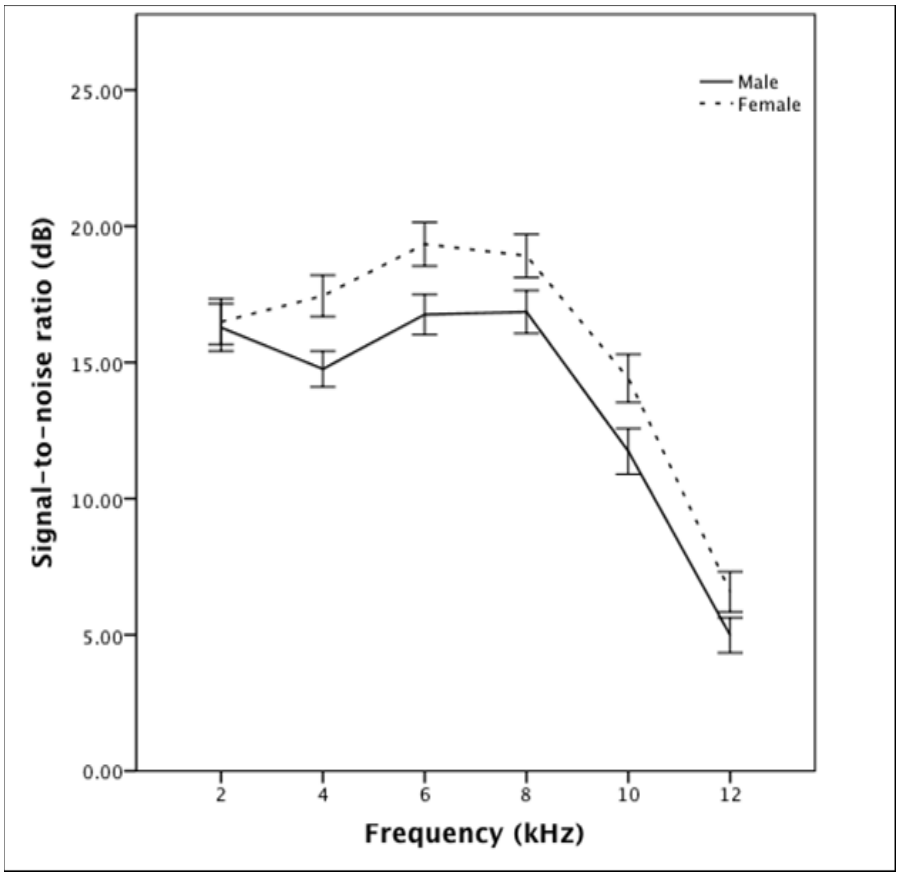

Figure 6-1: OAE signal-to-noise ratios (SNRs) for f_2 frequencies 2, 4, 6, 8, 10 and 12 kHz, for males and females. Error bars are SEM.

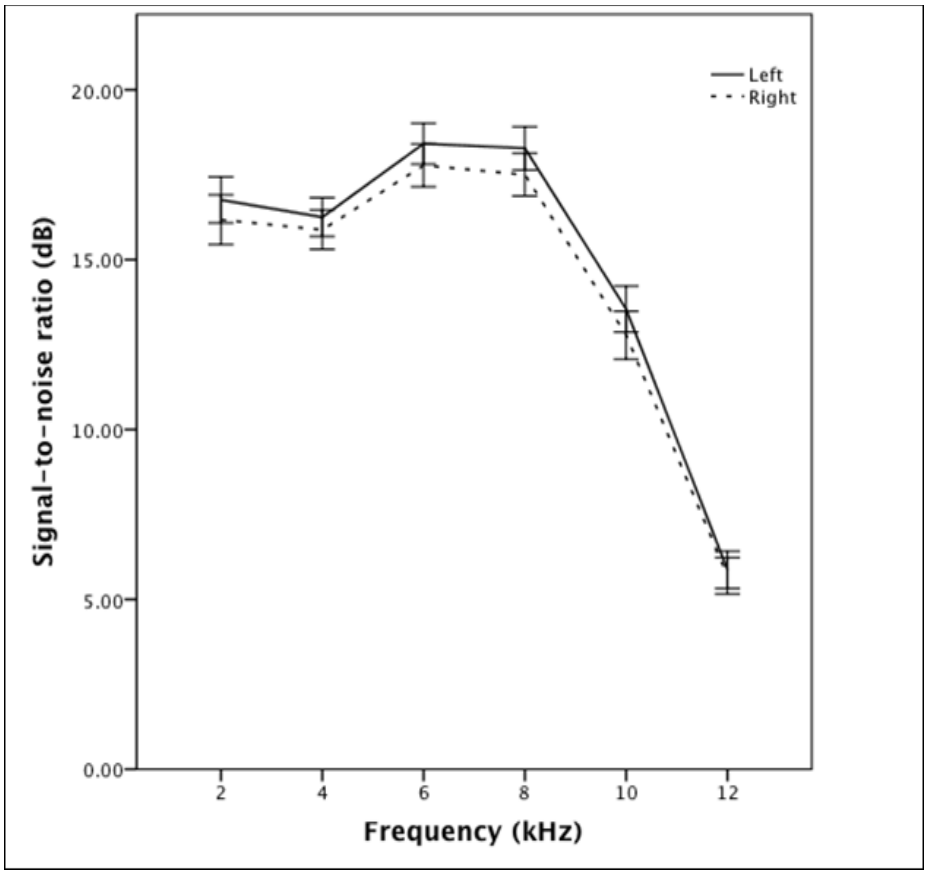

Figure 6-2: OAE SNRs for right ears and left ears. Error bars are SEM.

Figure 6-3 shows the SNR results of the high-frequency OAE test grouped according to whether the newborns (i) passed the initial OAE test, (ii) failed the initial OAE test but passed the AABR test, or (iii) failed both the initial OAE test and the AABR test. Based on our SNR cut-off value of 6 dB at individual frequencies, all of the newborns who passed the initial OAE test also passed the high-frequency OAE test at all frequencies except 12 kHz, where only 95 out of 224 had an SNR of at least 6 dB. Of the 31 (12%) who failed the initial OAE screening test, 23 passed the second-stage screening with AABR; all of these 23 newborns also had sufficiently high SNRs to pass at the high-frequency OAE test at f_2 frequencies of 4, 6 and 8 kHz, and all but

one passed at 10 kHz. The newborns who failed both the initial OAE test and AABR also failed the high-frequency OAE test at all frequencies with the exception of one newborn who passed only at 8 kHz. The failure rate and the false-positive rate for the initial OAE test were 12% (31/255) and 9% (23/247) respectively. Requiring an SNR of 6 dB or more at all of 4, 6, 8 and 10 kHz for a pass, our high-frequency OAE test led to a reduction of the OAE failure rate from 12% to 3.5% (9/255), and a reduction of the false-positive rate from 9% to 0.4% (1/247). If the SNR was required to exceed the threshold only at three of the four frequencies, the OAE failure rate would be slightly lower (8/255) and the false-positive rate would be zero. The mean SNR values were statistically different for the three groups across frequencies 2, 4, 6, 8 and 10 kHz ($p < 0.05$).

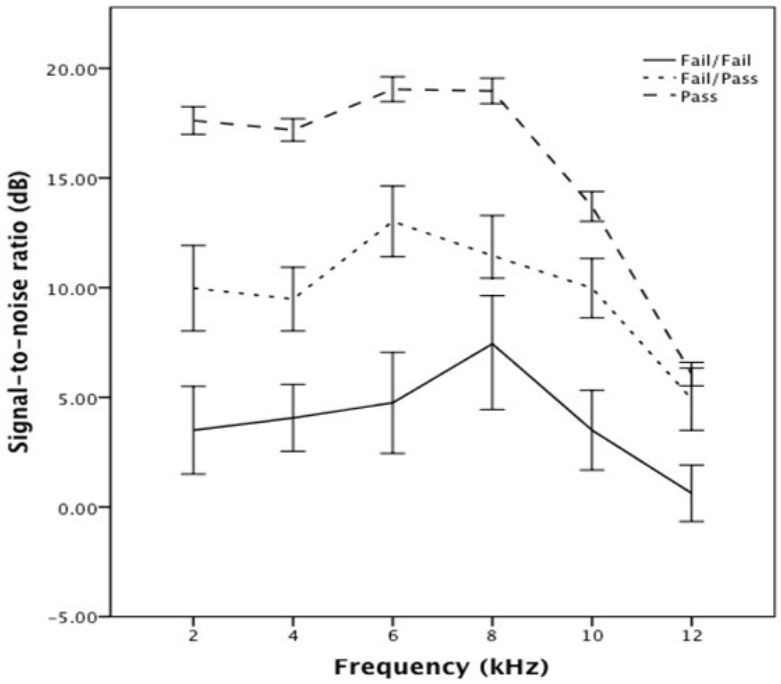

Figure 6-3: SNRs for the high-frequency OAE measurements grouped according to the performance of the newborn at the initial (conventional) OAE screening test: (i) those who passed the initial OAE test, (ii) those who failed the initial OAE test but passed the AABR test, and (iii) those who failed both the initial OAE test and the AABR test. Error bars are SEM.

Effects of birth weight, gestational age and mode of delivery

Table 6-1 contains the SNR values for the different subgroups according to the sex, mode of delivery, birth weight and gestational age.

Figure 6-4 shows the SNR values grouped by birth weight. A significant main effect was observed for the birth weights of the newborns ($F(4,249) = 4.3$, $p = 0\ .002$, $\eta^2 = 0.07$), and there was also a significant birth weight by frequency interaction effect ($F(20,1245) = 1.71$, $p = 0.03$, $\eta^2 = 0.03$). Post-hoc comparison of the birth-weight main effect, using between-group *t*-tests, showed that newborns with birth weights in the range 2500 to 2999 g had on average

significantly greater OAE SNRs than those with weights in the range 3500 and above ($t = 3.2$, $p = 0.002$, $d = 0.79$). SNR values were significantly higher for small babies (birth weights between 2500 and 2999 g) than for babies weighing 3500 g or more ($t = 3.60$, $p < 0.001$, $d = 0.63$). In addition, babies with birth weights ranging from 3000 to 3499 g had significantly higher SNR values than babies whose birth weights were from 3500 to 3999 g ($t = 3.43$, $p = 0.001$, $d = 0.92$).

Frequency (kHz)	Sex						Delivery type					
	Male (n=138)		Female (n=117)		Total (n=225)		Vaginal (n=160)		Cesarean (n=95)		Total (n=255)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	16.3	10.2	16.5	9.0	16.4	9.7	16.6	10.0	16.0	9.1	16.4	9.7
4	14.8	7.7	17.4	8.2	16	8.0	16.4	8.1	15.3	7.9	16.0	8.0
6	16.8	8.7	19.3	8.7	17.9	8.8	18.4	8.5	17.2	9.3	18.0	8.8
8	16.9	9.3	18.9	8.6	17.8	9	18.3	8.8	16.9	9.3	17.8	9.0
10	11.7	9.9	14.4	9.6	13	9.8	13.1	9.5	12.7	10.3	13.0	9.8
12	5.0	7.5	6.6	8.0	5.7	7.7	5.7	7.5	5.7	8.2	5.7	7.7

	Birth weight (g)											
	<2499 (n=9)		2500 – 2999 (n=51)		3000 – 3499 (n=83)		3500 – 3999 (n=86)		>4000 (n=25)		Total (n=254)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	19.2	9.3	17.9	10.4	16.8	9.3	16.1	9.4	11.8	9.7	16.4	9.7
4	17.1	8.7	18.0	8.2	16.3	7.7	15.1	8.0	13.4	8.5	16.0	8.0
6	19.1	9.3	18.4	8.3	19.1	8.8	17.1	9	15.8	9	17.9	8.8
8	17.9	11.2	20.8	8.9	19.4	8.5	15.3	8.3	14.5	9.6	17.7	8.9
10	15.9	12.3	16.9	10.0	14.3	9.2	9.9	9.3	9.4	7.8	12.9	9.8
12	6.6	7.8	8.7	9.0	6.1	7.3	4.4	7.1	2.0	5.8	5.7	7.7

	Gestational age (weeks)											
	37 (n=35)		38 (n=52)		39 (n=79)		40 (n=64)		41 (n=25)		Total (n=255)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	17.4	8.5	17.2	9.5	15.9	10.2	17.0	9.7	13.5	10	16.4	9.7
4	17.5	6.7	16.0	6.5	16.0	9.0	15.4	8.6	15.4	8.0	16.0	8.2
6	19.8	7.5	17.0	7.7	17.6	10.0	17.8	8.9	18.8	8.1	18.0	8.8
8	20.7	8.8	16.5	7.8	17.5	9.8	18.0	8.7	16.8	9.5	17.8	9.0
10	15.3	9.1	11.8	9.7	12.6	10.3	13.5	9.7	11.8	9.8	12.9	9.8
12	6.1	7.2	4.1	6.5	6.2	8.2	6.0	8.3	6.5	8.0	5.7	7.7

Table 6-1: Mean SNR values at the different frequencies, grouped by sex, delivery type, birth weight and gestational age.

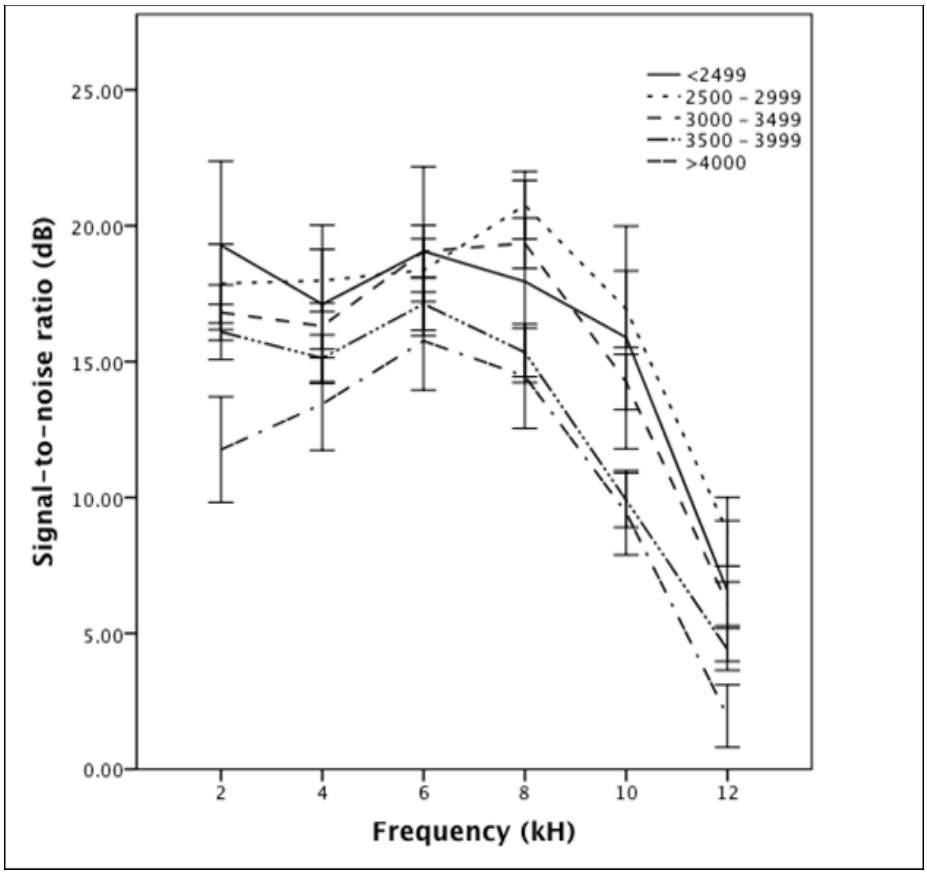

Figure 6-4: OAE SNRs grouped by birth weight. Error bars are SEM.

There were no statistically significant differences in the SNR values, either for the main effect of gestational age ($F(4,250) = 0.69$, $p = 0.60$, $\eta^2 = 0.011$) or for the gestational-age-by-frequency interaction effect ($F(4,250) = 1.09$, $p = 0.36$, $\eta^2 = 0.017$). However, the SNR values appeared to be highest for babies born at 37 weeks and lowest for babies born at 41 weeks.

Newborns delivered by Caesarean section had slightly lower SNR values than those who were born vaginally, but this difference did not reach statistical significance ($F(1,253) = 0.75$, $p = 0.39$, $\eta^2 = 0.003$).

No significant differences in SNR values were observed with respect to age at screening. Neither the main effect ($F(1,241) = 0.42$, $p = 0.66$, $\eta^2 = .003$) nor the age-by-frequency interaction effect ($F(2,241) = 1.0$, $p = 0.37$, $\eta^2 = 0.008$) reached significance. However, when other variables (birth weight, gestational age, mode of birth delivery) were taken into consideration, the age at screening had a significant effect ($\chi^2(6, N = 23) = 18.2$, $p = 0.006$) only at 2 kHz for the group of newborns who failed the initial OAE test but passed the AABR test; at other frequencies there were no significant effects.

OAE amplitudes and noise floors

Figure 6-5 shows the OAE amplitudes and noise floors for the newborns grouped according to their pass or fail status at the conventional OAE screening test. The OAE amplitudes were higher in the newborns who had a pass status than in those who had a fail status ($t(253) \geq 2.767$, $p < 0.05$) except at 2 and 12 kHz ($t(253) \leq 1.77$, $p \geq 0.08$). The noise floor at 2 kHz was significantly higher in the group of newborns who failed the initial OAE screening test than in those who passed it ($t(253) = 5.822$, $p = 0.02$), but at f_2 frequencies above 2 kHz the noise floors were similar for both groups ($t(253) \leq 1.8$, $p \geq 0.396$). The noise floors were not significantly different between sexes, nor for different gestational ages or birth weights.

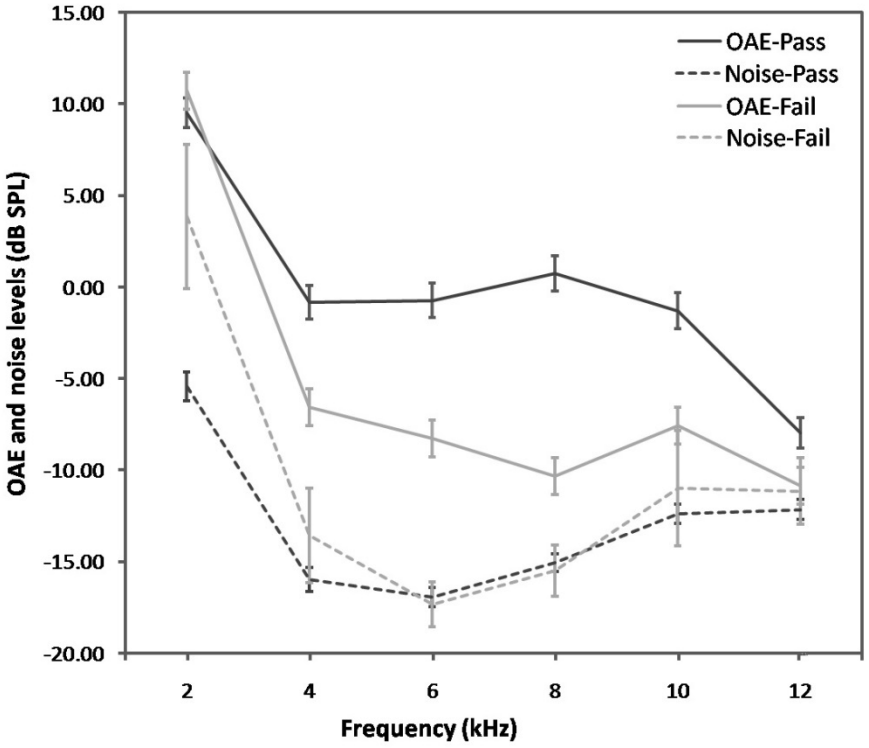

Figure 6-5: OAE amplitudes and noise floors in the groups of newborns with pass or fail status at the initial (conventional) OAE test. Error bars are SEM

Discussion

The current study demonstrates the presence of robust OAEs in healthy newborns up to 10 kHz. OAEs were consistently present at 2, 4, 6, 8 and 10 kHz in all the newborns that passed the initial (conventional) OAE screening test but they were not always present at 12 kHz. The SNRs generally became smaller at 10 and 12 kHz – only one third of the study population had SNRs that were high enough for a pass at 12 kHz. This is consistent with the findings of Dreisbach et al., (2005; 2006), who reported smaller amplitudes of OAEs at frequencies between 12 and 16 kHz in an older population. Due to the fact that high-frequency hearing is usually

better in younger people, and in particular in infants (Reuter et al. 1997; Werner & Boike 2001; Northern & Downs 2002), testing high-frequency OAEs in newborn hearing screening appears to be an area worth looking into. It might be expected that high-frequency OAEs would have higher amplitude in newborns than what has been previously reported for young adults; it is possible, however, that the relatively low levels that we report, especially at 12 kHz, are due to the fact that high-frequency OAEs are still developing in the perinatal period. Studies have shown that low frequencies are developmentally ahead of high frequencies (Graven ad Browne, 2008). Further studies on newborn high-frequency OAEs are required to improve our understanding of this point.

Our results showed that high-frequency OAEs were slightly (but with statistical significance) greater for the female newborns and for the right ears but noise floors were not statistically different for the two sexes or for the left and right ears. The differences with sex have been described previously for conventional OAEs up to 6 kHz (e.g. Gaskil & Brown 1990; Lonsbury-Martin et al., 1991), for frequencies up to 8 kHz but not above 9 kHz (Dunckley & Dreisbach, 2004), and for frequencies up to 13 kHz (Bowman et al., 2000); the reasons behind this finding are still uncertain. It has been suggested that ear-canal anatomical differences between male and female newborns may account for the observed sex difference (Ismail & Thornton, 2003): with females having a longer and thinner external auditory canal and presenting a smaller volume than males, low-level OAEs may become larger and easier to detect. Other studies have attributed this finding to the better auditory thresholds in females (McFadden, 1993) and to the fact that the basilar membrane is longer in males than in females (Sato et al., 1991; Kimberly et al., 1993). With respect to the left-right difference, it has been shown that the medial olivocochlear bundle, which innervates the outer hair cells that generate OAEs, is more active in

right ears than in left ears in adults (Kei et al. 1997; Ismail & Thornton, 2003). Further studies are required to clarify the effects of sex and side (right/left) on high-frequency OAEs, particularly in newborns.

We found that, for the frequencies we tested, newborns with a gestational age of 37 weeks had the highest SNR values; those at 41 weeks had the lowest SNR values, albeit still sufficient for a pass [*Figure 6-6 in the Appendix*]. These findings are not in complete agreement with those of Smolkin et al. (2013), who found that early-term newborns (born at 37 weeks) failed hearing screening tests more often than those with gestational ages between 38 and 41 weeks. They concluded that early-term newborns should be screened after 42 hours. In our population, those born at 37 weeks who were screened within 42 hours of birth did pass both the conventional OAE test and the high-frequency OAE test if they were born vaginally. Bonfils et al. (1992) reported that gestational age indeed had no effect on the amplitudes of OAEs, but they did not take into consideration the mode of delivery.

In our study, newborns delivered by Caesarean section had slightly (but statistically significantly) lower SNR values than those born vaginally [*Figure 6-7 in the Appendix*]. Analysis of the interactions among the different variables offered no explanation for this. Our observation corroborates a previously reported finding of greater failure rates on first OAE hearing tests being associated more with Caesarean births than with vaginal births (Smolkin et al. 2012). The reason for this difference was thought to be linked to the presence of retained liquid in the middle ear of the newborns. We also observed that, among those delivered by Caesarean section, newborns who weighed more than 3500 g had lower SNR values than those born at the same gestational ages but weighing less. The role of birth weight in newborn hearing screening has scarcely been studied and is poorly understood. Ari-Even Roth et al. (2006) found that very-low-

birth-weight babies had a lower prevalence of sensorineural hearing loss than other babies from the Neonatal Intensive Care Unit, and Christobal and Oghalai (2008) found that very low birth weight alone was not a risk factor for hearing loss in newborns.

The initial OAE test for our newborn population had a failure rate of 12% and a false-positive rate of 9%, taking the AABR result as the truth. Several factors have been shown to contribute to high OAE referral rates in newborns, including the presence of occluding ear-canal debris and transient middle-ear liquid (amniotic fluid) and mesenchyme (Chang et al. 1993; Thornton et al. 1993; Priner et al. 2003). Methods used previously to reduce false-positives include testing the newborns at a later age (e.g. Martinez et al. 2012); repeating the OAE tests once or twice before discharge from the birth admission (e.g. Govaerts et al. 2001); and use of two-staged screening with OAE and AABR (e.g. Govaerts et al. 2001). Additional testing at 4, 6, 8 and 10 kHz in this study resulted in significant reductions in the OAE failure and false-positive rates. A possible explanation for this is the fact that stiffness-dominated lower frequencies are more affected by conditions that increase the stiffness of the middle ear, as when there is liquid in the middle ear (Ravicz et al. 2004). In newborns who failed the initial OAE test but passed the AABR (representing the false-positive group), the outcome at 2 kHz was more likely to be a pass as the age at screening increased. This finding supports the observation that the performance at low frequencies is affected by transient conditions. Further studies comparing high-frequency OAEs with OAEs at 1 kHz may provide more insight into this.

We also found a significant difference in the noise floor at 2 kHz between newborns who passed and those who failed the initial OAE test. Popelka et al. (1998) demonstrated increased noise floors in certain middle-ear conditions. Noise floors in OAEs are important determinants of the signal-to-noise ratio, which in turn is used as the basis of the pass criterion in most OAE

devices. Noise floors are affected by endogenous factors (e.g. breathing movements), by environmental noise, and by the noise-identification algorithm of the OAE equipment. Conditions that increase the acoustic impedance of the middle ear are likely to also result in higher noise floors, especially at low frequencies (Akinpelu et al., under review). Since the occurrence of middle-ear liquid is one of the reasons for failures in OAE tests, it is not unlikely that the increase in the noise floor at 2 kHz demonstrated in our data is associated with middle-ear liquid.

One limitation of our study is the fact that we compared our high-frequency OAE test with AABR, which is another screening test with its own inherent problems. In addition, only babies who failed the conventional OAE tests underwent the AABR test, so the sensitivity and specificity of the high-frequency OAE test could not be ascertained. Future studies are therefore required to establish the ideal referral criteria and the specificity and sensitivity of the high-frequency OAE test. It is also worth mentioning that 3% of our newborns were referred for full audiological testing after the conventional DPOAE test and AABR. This is lower than the 4% benchmark recommended by JCIH (2007), so it might be argued that no improvement is necessary. However, our high-frequency OAE test reduced the number of newborns that required the AABR test, thereby leading to a reduction in the cost, the total time needed for the hearing screening, and unnecessary in-office follow-ups.

In conclusion, high-frequency OAEs are robust in newborns up to 10 kHz and newborns with normal hearing (according to AABR) will pass our high-frequency OAE test (except at 12 kHz), while newborns who have hearing loss (according to AABR) will fail our high-frequency OAE test. In our sample of newborns, high-frequency OAE measurements resulted in a reduction in the OAE failure rate and false-positive rate, which may prove helpful in universal newborn

hearing screening programs. Higher noise floors at lower frequencies, especially in those who failed the conventional OAE test, suggest that avoiding screening at such low frequencies may improve the outcomes of OAE screening programmes. Further studies on high-frequency OAEs in larger newborn populations are required.

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Appendix to Chapter 6

The following figures were not included in the manuscript corresponding to this chapter.

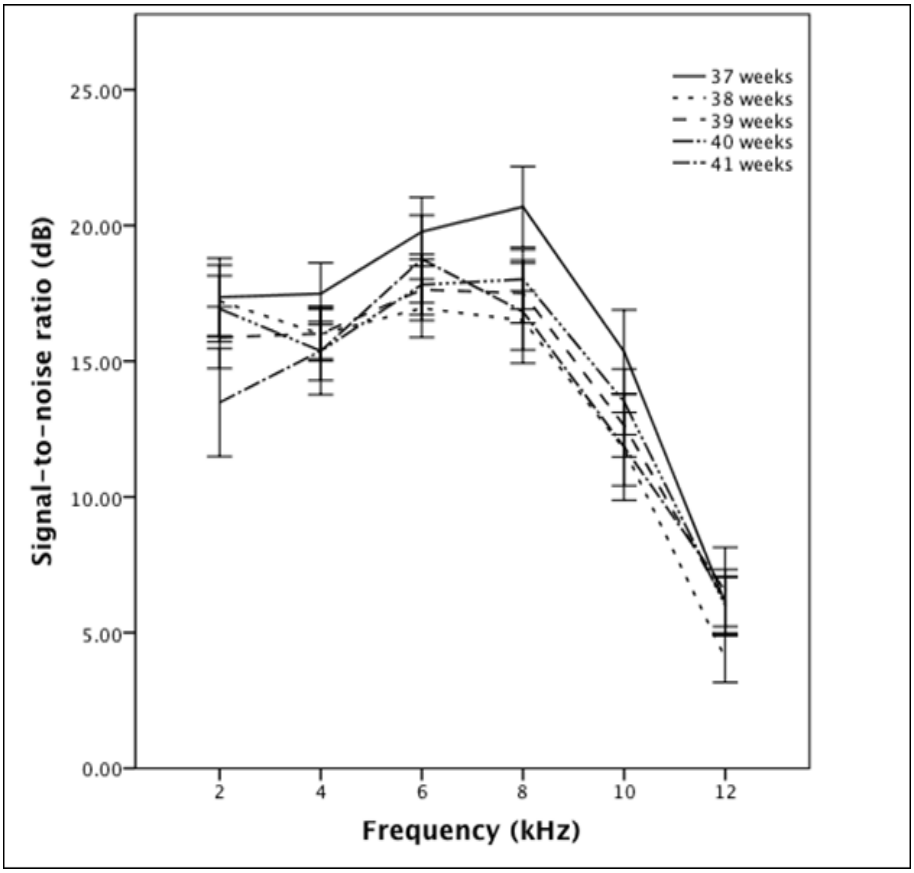

Figure 6-6: OAE SNRs grouped by gestational age.

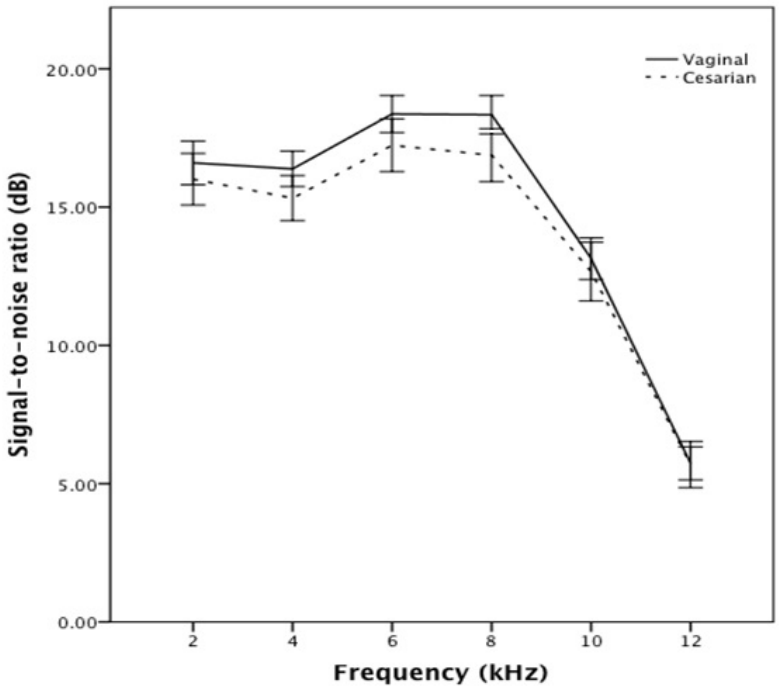

Figure 6-7: OAE SNRs grouped by mode of delivery.

CHAPTER 7 Conclusions and future work

Summary

The main issues that have been addressed in this research are the ways in which high-frequency testing affects the outcome of the OAE test and how this knowledge may be utilized to reduce false-positive results associated with OAE hearing screening tests in newborns. The systematic review (Chapter 3) established that high referral rates and false-positive rates from OAE screening were associated with screening within the first 24 to 48 hours of birth and with screening for lower frequencies. Multiple screenings before discharge from the hospital and screening for higher frequencies were associated with lower referral rates and false-positive rates. The empirical studies in Chapters 4 and 5 showed differences in OAE outcomes for high and low frequencies when there is middle-ear liquid, particularly with reference to noise floors. These differences to a large extent were replicated in the clinical study presented in Chapter 6.

It was established with the chinchilla animal model (Chapters 4 and 5) that noise-floor increases at low frequencies compromise the signal-to-noise ratio of OAEs generated at low frequencies and could make a difference between a pass and a fail status on the test. Middle-ear liquid gave rise to critical changes in the noise-floor levels at the three lowest frequencies tested. Higher frequencies (greater than or equal to 4 kHz), however, did not demonstrate this drawback. It was also shown that low-frequency OAEs were smaller in amplitude than the high-frequency OAEs and that they became submerged in the higher noise floors that occurred as a consequence of the presence of middle-ear liquid. It was already known that noise floors are greater at low frequencies than at high frequencies (Aidan et al., 1997); in this dissertation (Chapter 5) it was shown that noise-floor levels were increased not only with middle-ear liquid but also with

endogenous noise and decreasing cavity volumes. These increases were found to be higher when measurements were taken in a non-sound-treated room than when measurements were taken in a sound-treated room. In addition, we showed that changes occurring in OAE detection patterns were similar to those occurring in middle-ear function as examined with wideband energy-reflectance measurements.

In Chapter 6, high-frequency OAEs were shown to be present in all newborns who were true positives as determined by the AABR; this means that they were also present in the false-positive category (those who failed the conventional OAE test but passed the AABR test). Therefore, screening at those high frequencies greatly reduced the false-positive rate in this study. Noise floors were also shown to be higher at 2 kHz in this group of newborns.

Implications of results for universal newborn hearing screening

A universal newborn hearing screening program should have high sensitivity and specificity and should be affordable. The work in this thesis suggests that the use of high-frequency OAE screening in newborns (using frequencies from 4 to 10 kHz) could be a strategy for achieving an acceptable sensitivity and specificity for newborn OAE hearing screening tests. The immediate advantage, as has been discussed in this thesis, is the reduction of false-positive rates without producing false-negative rates different from what has been reported with the use of conventional OAEs at frequencies between 1 and 4 kHz.

Another advantage of using higher frequencies would be in the detection of high-frequency hearing loss. By focusing only on the low frequencies, important information relating to high-frequency hearing in newborns may be missed. This will be of particular benefit to babies in the neonatal intensive care unit, who are prone to high-frequency hearing loss because of exposure to ototoxic agents.

An alternative approach to reducing false-positive and referral rates is to screen for hearing loss at a later age (e.g., Smolkin et al., 2013). By delaying the screening by a few more days, transient conditions affecting the hearing screening are given time to resolve. However, the logistics of bringing all babies back for hearing screening makes this suggestion problematic. Modifying the OAE test to achieve lowered false-positive rates may be preferred to screening newborns at later dates, to avoid follow-up attrition. Current trends are in favour of shorter post-delivery hospital stays, so the provision of a tool which is effective within 48 hours is preferable.

Limitations and future work

We showed that human amniotic fluid has a shear-thinning behaviour which is similar to what obtains for most biological fluids. We showed that with low shear rates viscosity was high, and it dropped as the shear rates increased. This somewhat mirrors the findings that noise floors at low frequencies are greater with middle-ear liquid. It is therefore desirable to study the relationship between the shear rate and the sound frequency. This would further enhance our understanding of viscosity effects on middle-ear function and in particular on OAE detection.

The newborn data presented in Chapter 6 were limited in some respects. The randomization technique adopted was such that data were collected only on one day of the week; as such it was not possible to totally rule out selection bias, so future studies on high-frequency OAEs in newborns should adopt a proper randomization technique to ascertain the validity of this finding. Another limitation was that newborns who passed the OAE tests were not tested with the AABR test, so it was not possible to determine the sensitivity and specificity of the high-frequency OAE tests described in this study. Further studies are therefore required in which newborns will be tested with both the conventional OAE and the high-frequency-OAE tests and in addition will be tested with AABR. Also, the false-positive cohort in this study included only

23 babies. Having a larger sample size would presumably increase the size of the false-positive cohort and better validate the benefit seen with the use of high-frequency OAEs.

Another question raised by the results presented in Chapter 6 concerns the possible relationship between the noise-floor increase at 2 kHz and the middle-ear status in the group of newborns who failed the conventional OAE test. Although we showed that noise-floor levels were greater at 2 kHz than at 4, 6, 8, 10 and 12 kHz, and that this affected the SNR values obtained at this frequency, the exact aetiology of this noise-floor increase is still unclear. In Chapters 3 and 4 it was shown with an animal model that middle-ear liquid gave rise to increases in noise floors at low frequencies. It is therefore desirable to ascertain if middle-ear dysfunction was responsible for the observed noise-floor increases in the newborn study. It may be possible to answer this question by assessing the newborn's middle-ear function with WBR in addition to testing for low and high frequency OAEs; by so doing, middle-ear functional status can be compared with OAE amplitudes and noise-floor levels.

It has been suggested that, since OAEs depend to a great extent on the middle-ear transfer function, they might be useful in assessing the status of the middle-ear (e.g., Olzowy et al. 2010). In this dissertation it was shown that high frequencies are little affected by the presence of middle-ear liquid. It may be possible to utilize this knowledge to make OAEs a tool for testing the middle ear, by developing an algorithm that simultaneously assesses both the low-frequency and high-frequency OAEs and estimates the middle-ear status, as outlined in Table 7-1. This could be added to the current hearing-screening components of UNHS and would mean that middle-ear liquid in newborns could be detected, followed up and treated early.

Outcome	Low frequency	High frequency	Status
	Pass	Pass	Good
	Pass	Fail	Suspected HL
	Fail	Pass	Suspected ME problem
	Fail	Fail	Suspected HL

Table 7-1 Proposed use of both high- and low-frequency OAEs as a screening tool for middle-ear function assessment

Results presented in Chapter 6 showed that OAEs were not always present at 12 kHz in our newborn population. It is still not clear how and why OAE test outcomes at 12 kHz were different in the newborns studied. Birth weight was shown to play a role. A prospective cohort study assessing the effects of body weight on the performance at 12 kHz is desirable to enhance our understanding of high-frequency OAEs.

In conclusion, the major finding discussed in this dissertation was that of increased OAE noise-floor levels at low frequencies, and how this is made worse by certain middle-ear conditions. The influence of this on the outcome of newborn hearing screening is substantial and can possibly be corrected by screening at high frequencies.

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