The effect of estrogen and progesterone on sex differences in susceptibility to noise induced hearing loss.

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Abstract

There is some evidence suggesting that males and females differ in susceptibility to noise induced hearing loss (NIHL): that is, they differ in NIHL magnitude even when exposed to the exact same noise exposure (1, 2), and that this may be related to the effects of circulating levels of the female sex hormones estrogen and progesterone on the cochlear response to noise (3-8). The main objective of this research was to determine what effect estrogen and progesterone levels had on sex differences in susceptibility to human temporary threshold shift (TTS) and otoacoustic emission (OAE) shift. A secondary objective was to determine whether estrogen and progesterone levels impacted on the prediction of susceptibility to NIHL using measures of auditory physiology: OAE amplitude, efferent suppression magnitude and 4 kHz pure tone audiometry thresholds. Additionally, it was determined whether the female sex hormones acted to influence susceptibility to NIHL via their effect on these measures of auditory physiology or whether hormones acted independently of these effects to influence susceptibility to NIHL.

25 female and 21 male participants aged 18-35 were exposed to a 3 kHz, continuous, pure-tone noise exposure at 100 dB L_{Aeq} for 15 minutes in their right ear. This exposure provided the equivalent energy to an eight-hour continuous A-weighted sound pressure level, $L_{Aeq,8h}$ of 85 dB. To address the main objective TTS, OAE shift and recovery from TTS and OAE shift were compared in males and females. Serum levels of estrogen and progesterone were measured in female participants and correlations were made between these levels and TTS and OAE shift data. To address the second objective correlations were calculated between auditory physiology measures, TTS and OAE shift for males and females as well as between the sex hormones and auditory physiology measures. Additionally, linear regression models were produced to assess the mediating role of the auditory physiology measures on the relationship between hormones TTS and OAE shift.

This research found no difference between males and the entire group of females in susceptibility to TTS, OAE shift or recovery from OAE shift, although females had a slower recovery from TTS. However, when circulating levels of estrogen and progesterone levels were accounted for a sex difference in TTS was apparent. This

difference was driven by a large, significant, negative correlation between progesterone levels and TTS, whereas estrogen had no significant correlation with TTS or OAE shift. However, estrogen mediated different aspects of auditory physiology, whereas progesterone did not. There was no interaction between the effects of estrogen and progesterone on TTS or OAE shift. Additionally, there was a mediating role of some aspects of auditory function on the effects of estrogen on TTS and to a greater degree on OAE shift. However, estrogen itself only had a small non-significant impact on TTS and OAE shift so this suggests that the impact of auditory function and hormones on TTS and OAE shift are independent.

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Introduction

Excessive noise can cause injury to the delicate structures of the cochlea which can lead to a noise induced hearing loss (NIHL) (9, 10). Although the damaging effects of excessive noise are well understood (11), excessive noise in the workplace and in society in general continues to be a problem and consequently people continue to suffer from NIHL (11). In New Zealand it is estimated that NIHL accounts for between 13.5% and 17.5% of hearing loss (12). NIHL can cause significant personal costs for individuals in the form of impaired communication skills and the subsequent impact on personal, social aspects and employment aspects of their lives (13), as well as significant societal costs in terms of funding the costs of rehabilitation options for NIHL (11).

Males and females do not appear to have the same prevalence of NIHL. In New Zealand, estimates of NIHL in females vary from between 5% to 50% of the total amount of NIHL, however most evidence does suggest that prevalence is lower for females than males (14, 15). The 5% figure is an estimate based on the percentage of female notifications to the Department of Labour's Notifiable Occupational Disease (NODs) register (14). As this register is voluntary it underestimates the amount of NIHL (15). It is uncertain if, and to what degree it would accurately represent female NIHL relative to male. Other predictions are higher, such as 30-50% of the amount of male cases of NIHL (15). This is based on factors such as the difference in the percentage of male and females reporting that they are exposed to loud noise more than 25% of the time in their current job in the New Zealand workforce survey (15). Overseas data also indicates a difference in prevalence, with worldwide data indicating that 22% of disabling hearing loss in males is attributable to occupational noise exposure whereas in females the figure is 11% (16). The same pattern of a greater percentage of disabling hearing loss being attributable to noise exposure in males than females occurred in all regions of the world (16).

This data suggesting a difference in NIHL prevalence between males and females begs the question of what might underlie this difference? This may be due to

differences between males and females in the total lifetime noise exposure. Much of the burden of NIHL is due to occupational exposures (16) and male participation rates in the labour force are greater than female rates (15, 16) and male participation levels in industries that have high levels of NIHL such as agriculture, forestry and fishing and construction are higher than female participation levels (15, 16). Males also have higher levels of non-occupational noise exposure (17-22). For example, males tend to listen to music for longer periods and at louder levels. Males also have higher levels of firearm and tool use (17-22). To illustrate this point in the Epidemiology of Hearing Loss Study in Beaver Dam Wisconsin, 54% of females and 98% of males report having noisy hobbies and 1% of females and 31% of males report firearm usage (21).

However, there is little data on the relative risk of NIHL in males and females who are exposed to the same noise exposure (15). This main aim of this thesis is to establish whether there are sex differences in susceptibility to NIHL: that is, do males and females differ in NIHL magnitude even when exposed to identical noise exposures (1, 6). This thesis will use the terminology recommended by the Institute of Medicine Committee on Understanding the Biology of Sex and Gender Differences (23) which suggests the use of sex when classifying humans, "generally as male or female, according to the reproductive organs and functions that derive from the chromosomal complement" (23), p 437, and the use of gender when classifying humans according to their "self-representation as male or female, or how that person is responded to by social institutions on the basis of the individual's gender presentation" (23), p 437.

Becker et al (24) offer a set of experimental questions for investigation of the presence and origin of sex differences in susceptibility to external stressors which can be applied to the investigation of differences in susceptibility to NIHL in human males and females. They state that the presence of a sex difference is likely due to either differences in the action of circulating female sex hormones at the time of testing (activational effects) or to permanent differences caused by the action of sex hormones in the foetal stage (organisational effects) (24). The first stage is to establish whether there is a difference between males and females in

susceptibility to NIHL (Figure 1). This can be determined by measuring the differences in NIHL that occur in a chosen group of males and females. If no difference is found then one must consider the possibility of sex effects working in the opposite direction to cancel each other out before determining that there are no differences in susceptibility. This could include small differences in function that are apparent during some stages of the menstrual cycle and not at others. If a difference between males and females is found in susceptibility to NIHL then the second stage is to determine whether this is due to activational effects of hormones. This is done by measuring the hormones of interest at the time of testing. If no activational effect is found then this raises the question of whether there are organisational effects. If no organisational effects are found then sex chromosome effects should be ruled out before definitively stating that there is no sex difference (24).

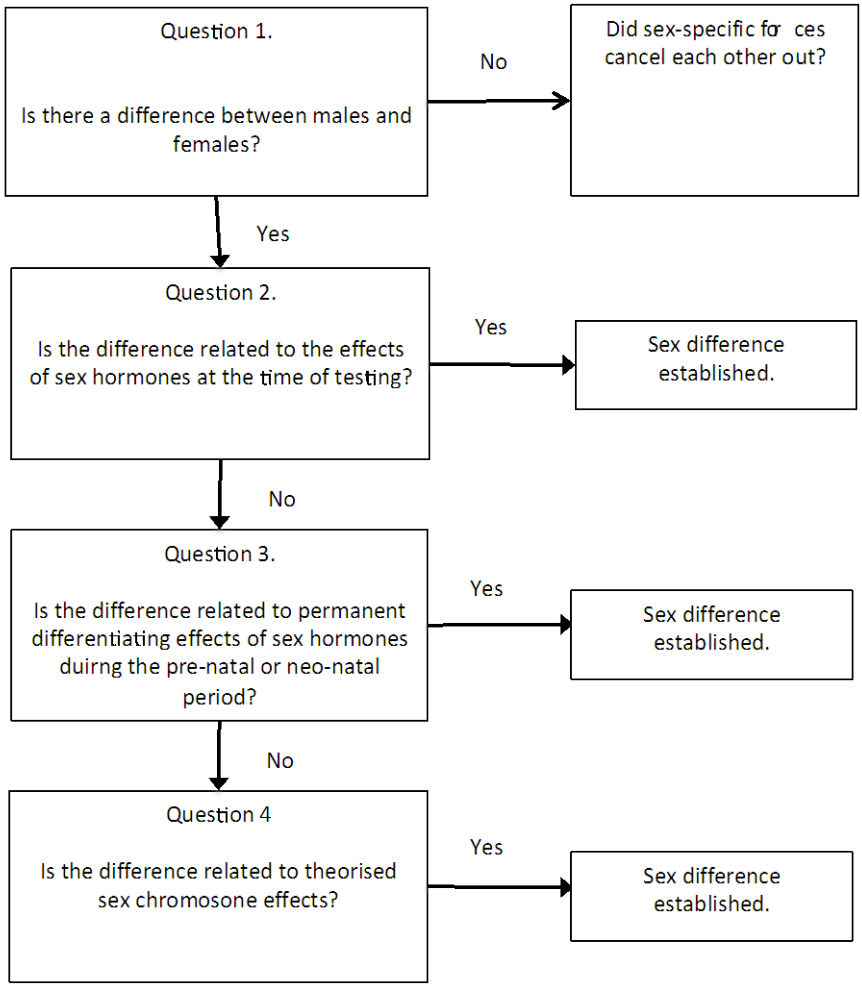


Figure 1: Guidelines for the investigation of the origin of sex differences in NIHL. Modified from (24), pg.1652.

This study seeks to answer questions one and two of Becker et al's set of experimental questions as there is some evidence suggesting that males and females might differ in susceptibility to NIHL (1, 2). Additionally there is some evidence that this may be related to the effects of circulating levels of the female sex hormones estrogen and perhaps progesterone on the cochlear response to noise (3-8). However, there is no data that has specifically looked at the association of measured circulating hormone levels and NIHL in humans. The presence of such an activational effect of hormones on NIHL would confirm the presence of a sex difference in susceptibility to NIHL as this is an effect that would occur in females and not in males (24).

A secondary aim of the thesis was to determine the effect of sex and circulating levels of estrogen and progesterone on the measurement of susceptibility to NIHL. There is a large range of individual susceptibility to NIHL, for example, the threshold shifts in group of jute weavers exposed to equivalent long term noise exposures ranged from 10 dB for the least susceptible to 70-80 dB for the most susceptible (25). This is the difference between a barely noticeable and very debilitating hearing loss. Attempts to accurately measure individual susceptibility to NIHL are on-going due to the need to be able to predict particularly susceptible individuals before their hearing has been damaged (26).

Various measures of auditory physiology have been found to be predictive of NIHL size: these are pure tone audiometry (PTA) thresholds and otoacoustic emission (OAE) based measures of auditory function including OAE amplitude and efferent suppression amplitude (2-4, 27-34). Sex differences have been proposed in these aspects of auditory function (35-47) and evidence suggests these sex differences may be related to circulating levels of the sex hormones estrogen and progesterone (3, 4, 48-50). Given that sex hormones may impact on both susceptibility to NIHL (1, 2) and on the strength of those measures that have been used to predict susceptibility (3, 4, 48-50) it is important to consider whether sex and circulating levels of female sex hormones are factors that need to be considered when determining the effectiveness of tests of NIHL susceptibility. Furthermore, it remains to be determined whether sex hormones act to influence

susceptibility to NIHL through their effect on these aspects of auditory function or whether hormones act independently of these effects to influence susceptibility to NIHL.

Knowledge gained from understanding the origin of sex differences in susceptibility to NIHL and of how female sex hormones impact on the measurement of susceptibility to NIHL will further understanding of the nature of NIHL susceptibility and may assist in the eventual development of a diagnostic tool that can be used to predict individual susceptibility to NIHL. Given that the aim of occupational noise management programmes is to prevent individuals from developing NIHL (11) it would be useful to have information about an individuals susceptibility. This information, in conjunction with the noise monitoring and control and audiological monitoring which are essential parts of an effective programme, could be used to prevent NIHL by accurately establishing individual risk and directing the prevention programme to account for this additional risk by providing such things as extra audiological monitoring, training and hearing protection for those who are particularly susceptible (51).

Literature Review

This chapter begins with a background section which will include a brief discussion of the basis of sex differences, estrogen and progesterone and the menstrual cycle which unless otherwise mentioned is based on the following reviews in (24, 52-54). This will aid understanding of the hormonal aspects of the thesis. It then provides some background information on the structure and function of the peripheral auditory system and the mechanisms of NIHL.

This literature review then examines the evidence for a sex difference in susceptibility to NIHL and whether this difference is related to the circulating effects of the female sex hormones, estrogen and progesterone. This will be followed by consideration of the measurement of susceptibility in NIHL using auditory physiology measures and whether there are sex differences in these auditory measures that are related to circulating effects of estrogen and progesterone

Background

The basis of sex differences

The basis of all sex differences is genetic: humans have 46 chromosomes, including two sex chromosomes, XY in males and XX in females. Sex differences are initiated by this imbalance of X and Y genes. The *Sry* gene that determines sex is located on the Y chromosome and causes the development of testes. The testes produce and secrete testosterone: the main male sex hormone. This causes the development of a male phenotype. In the absence of the *Sry* gene and the resultant testosterone, ovaries and a female phenotype develop.

There are two major types of gonadal (testes or ovaries) hormone action. Organisational effects are permanent and occur when hormones (testosterone) act on structures such as the genitals and brain in the foetus and neonate to cause a male phenotype resulting in permanently differentiated structures. Activational effects occur later in life when hormones act on these tissues. These effects are

changeable with the observable sex differences caused by differences in circulating levels of sex hormones at the time of measurement. Activational effects can be abolished by removing the adult gonads. Although these are the main causes of sex differences there is also evidence of sex chromosome effects or a non-hormonal cause of sex differences, with sex chromosome genes acting directly on non-gonadal structures to cause sex differences. Figure 2 illustrates these three known causes of sex differences. There is some evidence suggesting both activational and organisational hormonal effects in the cochlea (49, 55), while there are currently no reports showing either the presence or absence of a sex chromosome effect.

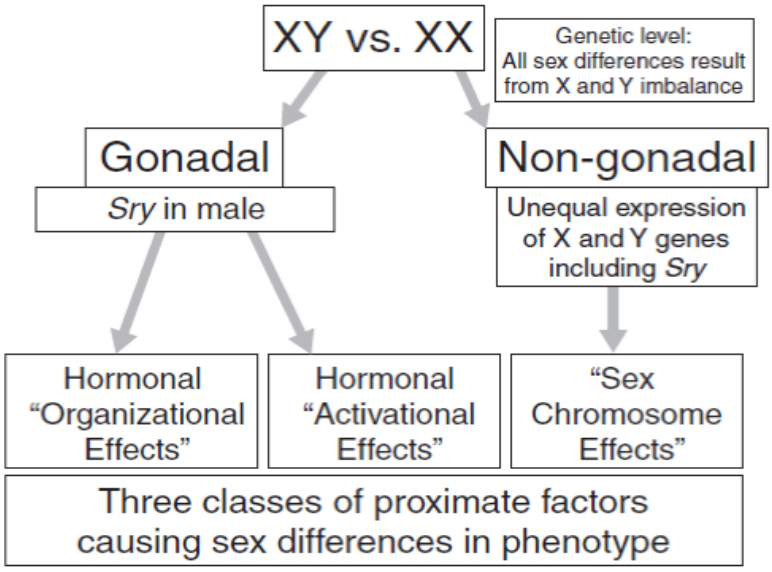


Figure 2: The three known causes of sex differences. From (54).

Estrogen and progesterone

In females the ovaries are responsible for the production and secretion of estrogens and progestins. The most potent type of estrogen is 17β estradiol (E_2) and the principal progestin is progesterone. Most estrogen is produced in the ovaries although there is some production in non-gonadal organs such as the brain, liver, fat and skin. This occurs through the conversion of testosterone via aromatisation into estradiol by aromatase. In males almost all estrogen is produced through the aromatisation of testosterone in non-gonadal tissues with 10-20% produced in the testes. Progesterone is produced in the male and female gonads and in the adrenal gland. Progesterone has an important role in pregnancy and is produced and released in large amounts by the placenta during pregnancy.

The action of estrogen and progesterone in cells is mediated through their interaction with receptors. The level of hormone receptors present in a cell is an important determinant of the cells response to a hormone. The factors that control receptor expression are not well known but probably depend on factors such as cell type, sex, developmental stage and exposure to sex hormones. The presence of a receptor in a cell is considered presumptive of an action of that hormone on that cell type. The estrogen receptor (ER) types are called ER alpha (ERα) and ER beta (ERβ). The progesterone receptors (PR) are termed PRA and PRB.

Sex hormones have been shown to have both genomic (slow) and non-genomic (fast) pathways of action (Figure 3). The genomic pathway is initiated when the hormone binds to receptors in the cell cytoplasm. The hormone/receptor complex then migrates to the cell nucleus and alters gene expression (mRNA). This pathway has a timeframe of hours or even days. Some hormonal actions are too fast to be mediated by this pathway and have been found to operate through the non-genomic pathway. This is initiated when hormones bind to receptors in the cell membrane. These receptors activate 2nd messenger signalling pathways that have rapid effects. This pathway has a timeframe of seconds or minutes.

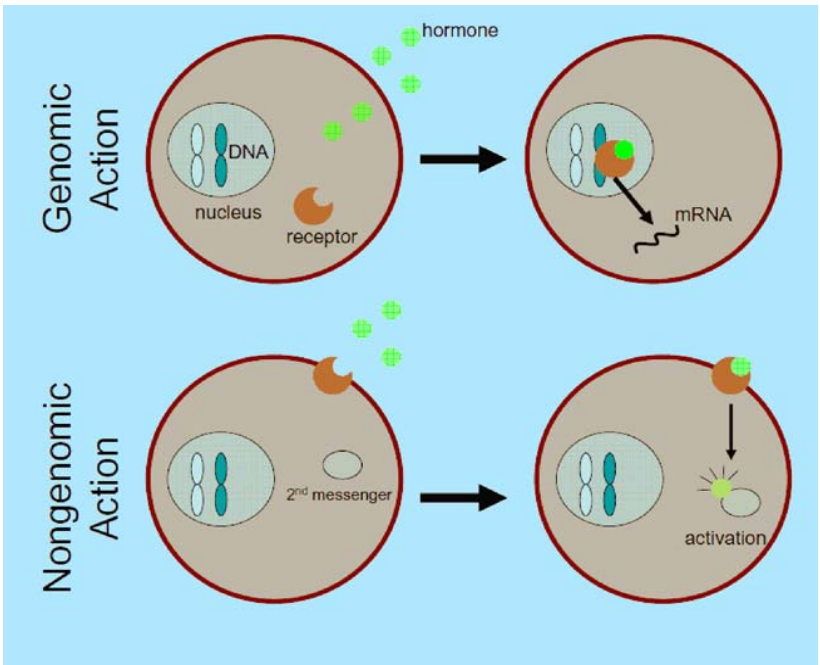


Figure 3: Illustration of the genomic and non-genomic pathways of estrogen and progesterone action. From (56).

The menstrual cycle

Circulating levels of estrogen and progesterone are higher and more variable in adult females than males; fluctuating throughout the menstrual cycle. The median length of the menstrual cycle is 29.5 days and normally-cycling women can have cycles of between 25-35 days. The events of the menstrual cycle are controlled by the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) that reach a peak at ovulation. The cycle begins on the first day of menses and can be divided into pre and post-ovulatory phases. The post-ovulatory (luteal) phase is reasonably constant in length at 13-15 days. The pre-ovulatory (follicular) phase is more variable and accounts for most of the variation in the length of the menstrual cycle. Estrogen levels are initially low during the early-follicular phase until ~7-8 days before the LH surge (Figure 4). In the late-follicular phase levels surge and reach a peak just before the LH surge. In the luteal phase there is a sharp drop in estrogen at days 14-16 of the cycle followed by a second rise to another peak at around 20-26 days.

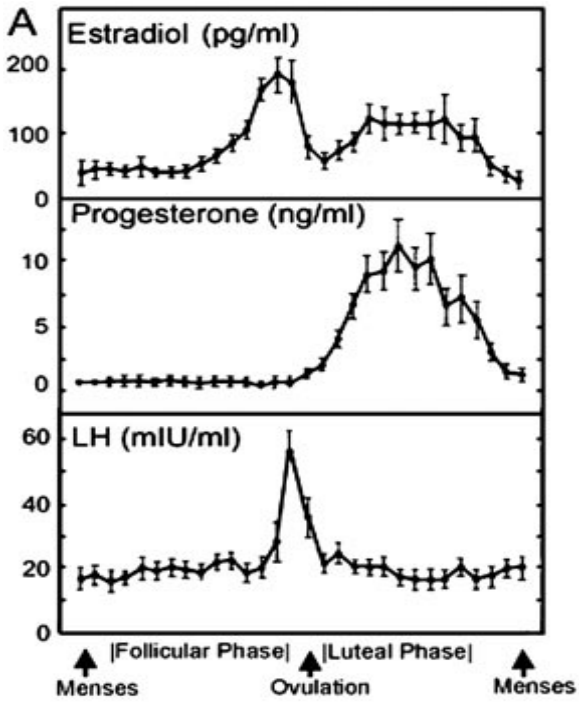


Figure 4: Illustration of the pattern of hormone changes during the female menstrual cycle. From (24).

Progesterone levels are low in the follicular phase and rise sharply in the luteal phase. The peak level is around days 18-24 of the cycle. Near the end of the cycle at

around day 24 both progesterone and estrogen levels decline. The concentration of estrogen and progesterone in males doesn't vary cyclically and is at or below that found in the early-follicular phase for females.

Structure and function of the peripheral auditory system

This section provides a brief outline of the basic structure and function of the auditory system, before discussing the nature and mechanisms of how the auditory system is damaged by noise. Unless otherwise mentioned details have been obtained from (57-60). The purpose of the auditory system is to detect and process sound and all the individual components of the auditory system play an important role in this process. The peripheral auditory system is divided into three parts: the outer, middle and inner ear. This division is illustrated below in Figure 5.

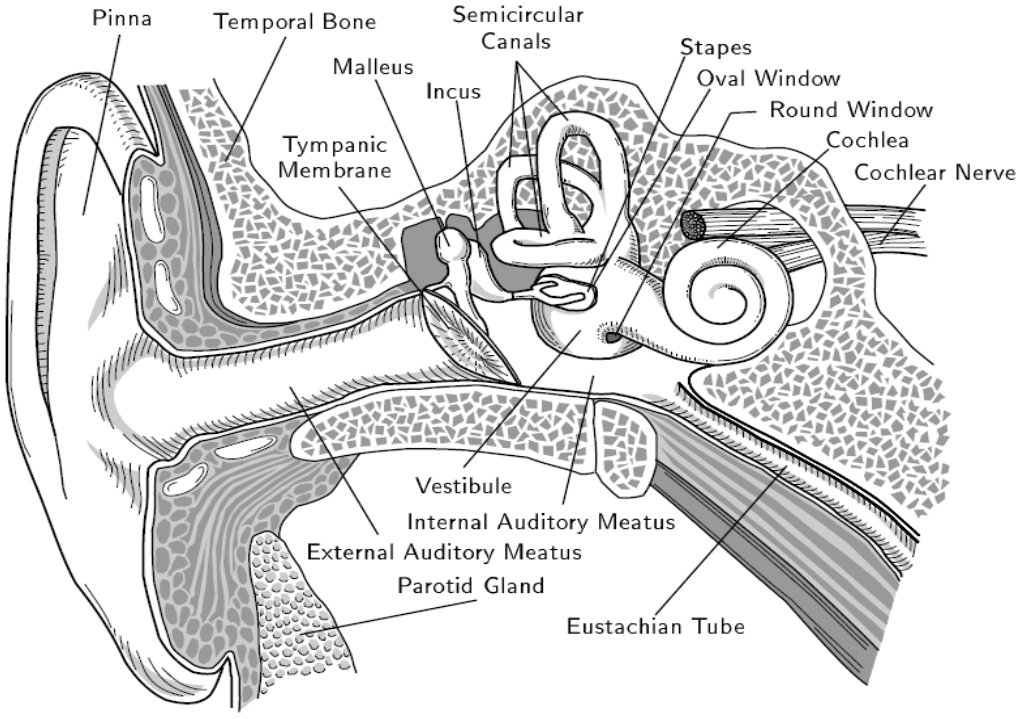


Figure 5: Illustration of the general organisation and components of the human peripheral auditory system. From (61). The outer ear consists of the pinna, external auditory meatus (ear canal) and tympanic membrane (ear drum). The middle ear contains the ossicles: the malleus, incus and stapes. The inner ear contains the vestibular apparatus and the cochlea. The auditory nerve inserts into the cochlea and transmits information to the central auditory system.

The outer ear consists of the pinna, external auditory meatus (ear canal) and tympanic membrane (ear drum). The pinna and external ear canal filter and funnel

sound energy to the ear drum. The sound energy is then transmitted by the middle ear ossicles (malleus, incus and stapes) to the cochlea of the inner ear via the insertion of the stapes into the oval window of the cochlea. The resonant properties and action of the outer and middle ear enhances transmission of some frequencies of sound, with the exact frequencies depending on individual characteristics.

The sound-induced movement of the stapes in the oval window of the cochlea causes a displacement of the fluid of the cochlea which causes a wave-like displacement of the basilar membrane upon which sits the organ of Corti: the receptor organ of the cochlea. This is situated within the scala media (Figure 6), which contains the structures necessary for transforming sound from mechanical energy into the electrical impulses which are transmitted by the auditory nerve to the higher auditory centres. The scala media also contains other important structures such as the stria vascularis and spiral ligament of the lateral wall which have important roles in maintaining transduction and in cochlear blood supply, as well as a number of support cells which support the structure of the organ of Corti and have other specialised functions.

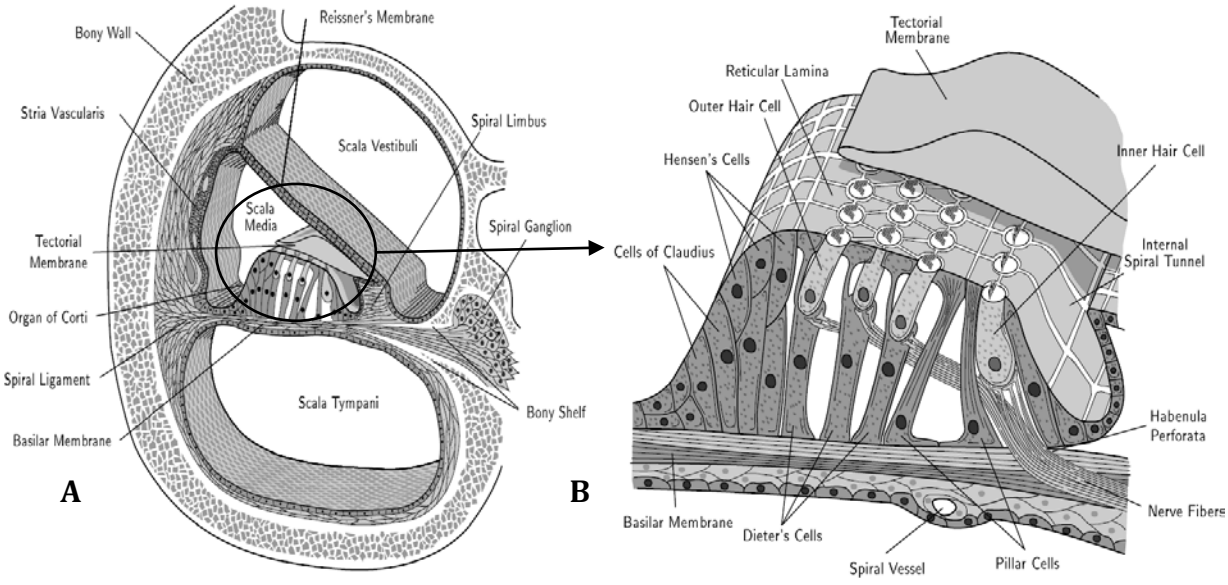


Figure 6A: Illustration of the major structures and organisation of the cochlea. From (61). The scala media contains the organ of Corti which is illustrated in more detail in Figure 6B as well as the stria vascularis and spiral ligament of the lateral wall.

This displacement is called a travelling wave, and moves from the base of the cochlea to a point of maximum vibration along the length of the basilar membrane. High frequency stimuli will lead to maximum basilar membrane vibration near the base and low frequency stimuli will cause maximum vibration towards the apex. Movement of the basilar membrane is also intensity related, with higher intensity sounds leading to larger movements.

The travelling wave causes mechanically gated transduction channels on the stereocilia of the one row of inner hair cells (IHCs) to open. Deflection of the stereocilia in an excitatory direction (towards the lateral wall of the scala media) allows entry of K^+ and Ca^{2+} ions which generates a transduction current. The transduction current activates voltage sensitive Ca^{2+} channels on the IHCs basolateral membrane and Ca^{2+} activated K^+ channels. This results in increased release of a neurotransmitter (glutamate) at the base, and stimulation of the auditory nerve. Transduction is made possible by the endocochlear potential (EP) (+80 mV), the positive electrical potential of the endolymph (which bathes the apical surfaces of the hair cells) relative to the perilymph of the rest of the cochlea and which is the main driving force for transduction (62). The EP is maintained by the stria vascularis and drives K^+ from the endolymph to the hair cells by its voltage gradient. This gradient is constantly maintained by the recycling of K^+ back into the endolymph.

The three rows of outer hair cells (OHCs) have a different response to sound than the IHCs. Their main response is a rapid change in the length and stiffness of the hair cell which is termed motility. The cell shortens and decreases the space between the tectorial membrane and reticular lamina when it is depolarised and lengthens increasing the space between the tectorial membrane and reticular lamina when hyperpolarised (63). These changes are generated by conformational changes in prestin, a motor protein located in the lateral wall of the OHC (64). This provides amplification of basilar membrane motion in a frequency specific region of the organ of Corti, feeding in energy before transduction is completed by the IHCs. This increases the sensitivity and frequency selectivity of the cochlea and is termed the cochlear amplifier.

Otoacoustic Emissions

As a by-product of the cochlear amplifier otoacoustic emissions (OAEs) are produced (65). These are low-level sounds that originate in the healthy cochlea from the action of the OHCs either spontaneously (SOAEs) or in response to an evoking stimulus and which propagate out from the cochlea and through the middle ear and into the ear canal where they can be measured by sensitive microphones (65).

The two main types of evoked OAEs are distortion product OAEs (DPOAEs) and transient evoked OAEs (TEOAEs). TEOAEs are generally measured in response to a brief and abrupt click stimulus. A click has a broad frequency range, which allows information to be obtained from a large part of the cochlea at the same time (65). DPOAEs are measured in response to the simultaneous presentation of two different pure tones (f1 and f2) which stimulate two different places along the cochlea (66). The interaction of the travelling waves caused by these two pure tones along the basilar membrane leads to the creation of a distortion product (DP) at another location along the basilar membrane that responds to a frequency that was not present in the two pure tones (67).

Although all OAE types rely on the underlying health of the OHCs their mechanism of generation differs. The mechanism behind TEOAEs and SOAEs is primarily a linear reflection based mechanism (68, 69). This mechanism results from the linear reflection of the forward travelling wave from randomly distributed impedance perturbations that are fixed in place along the organ of Corti. This may include such things as variations in hair cell number or spacing. Those reflections that add together constructively and arise from the tip of the travelling wave will be large enough to be recorded as emissions in the ear canal (69, 70).

The mechanism behind DPOAEs is primarily a non-linear distortion mechanism from the f2 place and a linear reflection mechanism from the distortion product place (68, 69). The distortion source mechanism results from the non-linearity of the cochlear amplifier (less intense sounds are amplified more than more intense sounds) (70). However, both mechanisms contribute to all forms of the OAE

measured in the ear canal with the proportion depending particularly on stimulus level. TEOAEs at low stimulus levels arise mostly from linear reflection, but at higher stimulus levels have a component of both linear reflection and non-linear distortion. DPOAEs have a larger component of linear reflection at lower stimulus levels (68-71).

The cochlear blood supply originates in the basilar artery of the brain stem. This supplies the anterior inferior cerebellar artery which supplies the spiral modiolar artery (SMA) which is located in the modiolus, the central bony core of the cochlea (see Figure 7). The SMA supplies capillary beds in the modiolus and then branches into radiating arterioles which supply the capillary beds in the stria vascularis and spiral ligament of the lateral wall (72, 73).

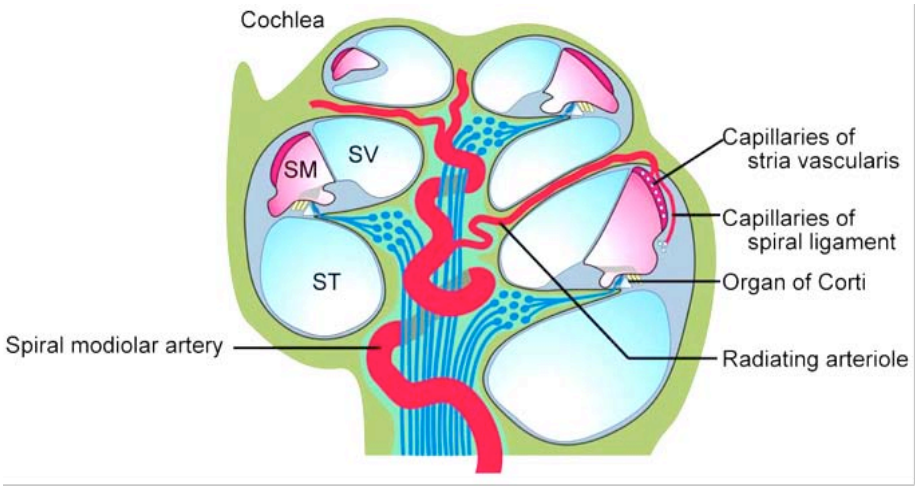


Figure 7: Illustration of the blood vessels supplying the cochlea. From (72).

The cochlea has both afferent and efferent innervation. The afferent nerve fibres of the auditory nerve originate in the cochlear nuclei (CN) in the brainstem and have cell bodies in the spiral ganglion of the modiolus. Most afferent fibres synapse with the IHCs and are called type I afferent fibres. Each IHC receives innervation from around 20 type 1 afferent fibres. While the IHCs receive most of the afferent innervation the OHCs receive the bulk of the efferent innervation. The efferent nerve fibres originate in the superior olivary complex (SOC) of the brainstem. The fibres project to the cochlea through two main pathways. Lateral olivocochlear system (LOC) fibres arise from cell bodies in the lateral SOC. There are relatively few LOC fibres and they mainly innervate ipsilateral IHCs terminating on the

dendrites of the type I afferent fibres rather than the IHCs themselves. Medial olivocochlear system (MOC) fibres arise from cell bodies in the medial SOC. These fibres mainly innervate the base of contralateral OHCs.

Once the neural response to sound is generated in the auditory nerve the signal is transferred to and processed by the CN. Fibres from the CN then project mostly contralaterally to the SOC and then via the lateral lemniscus to the inferior colliculus and the medial geniculate body to the auditory cortex in the temporal lobe.

As well as providing information to higher centres the cochlea receives efferent innervation and feedback from central auditory centres. This efferent pathway runs parallel to the afferent pathway from the auditory cortex back to the cochlea, with the most well studied pathway running from the MOC. Cochlear transduction of sound is effected (generally suppressed) by either electrical or acoustical stimulation of the efferent neurons (74). The reduction of cochlear sensitivity can be measured clinically in a reduction of the amplitude of measures of cochlear function and output such as OAEs and the compound action potential (CAP)(74).

Generally, efferent suppression occurs immediately upon presentation of the efferent stimulus and after the stimulus is removed suppression effects diminish and amplitudes quickly return to baseline levels (74). However, there are slow and fast efferent effects so called due to their time constants. Fast efferent effects occur within ~ 10-100 ms of the efferent stimulus onset and also have a fast offset (75, 76), whereas slow effects operate over a slower time scale ~ 10-100 s (76). Stimulation of efferent system causes the release of acetylcholine (ACh) from the synapse of the efferent fibres (77) which binds to α9 and α10 ACh receptors on the base of the OHC (78). This leads to an influx of Ca^{2+} from outlying cochlear fluids and also release of Ca^{2+} from sub-synaptic cisternae. Following this the fast and slow efferent effects are mediated through two separate pathways. On a fast time scale this increase in intracellular calcium causes activation of Ca^{2+} activated SK2 K^{+} channels that are at the base of the OHC opposite the ACh synapses. This causes an efflux of K^{+} and leads to hyperpolarisation of the OHC. This reduces the OHC

electromotile response and contribution to cochlear amplification leading to a reduction in basilar membrane motion (74, 76). If ACh application continues then the increased intracellular Ca^{2+} leads to Ca^{2+} release along the OHC membrane and Ca^{2+} activated modification of prestin and/or cytoskeletal proteins leading to a change in OHC stiffness (74, 76). This, as with the fast effect leads to a reduction in basilar membrane motion and decreased amplitude of cochlear responses (76).

Cochlear noise injury and hearing loss

NIHL occurs when excessive noise causes injury to the structure and function of the cochlea. The OHCs are the cochlear element most vulnerable to noise induced injury (10). This injury reduces the sensitivity of the auditory system and is manifested in humans as an increase in the threshold of hearing (hearing loss) as measured by PTA and/or as a decrease in the OAEs which are a measure of OHC function (79). Noise induced threshold shifts can vary in size and be either permanent (PTS) or temporary (TTS) depending on the severity of the noise exposure and on an individual's susceptibility to noise induced injury (25, 79). The issue of susceptibility to NIHL will be discussed in more detail in later sections.

The severity of a noise exposure is most often defined in terms of its intensity and duration. The equal energy hypothesis attempts to explain the relationship between the intensity and duration of a noise exposure and the subsequent noise induced cochlear injury and hearing loss. It states that equal amounts of noise exposure will produce equal amounts of damage: that is, a noise exposure of two hours will be equally as damaging as a noise exposure of 4 hours that is half its intensity and for noise of given intensity a 2 hour exposure will be twice as damaging as a 1 hour exposure (79). In the dB scale in which sound intensity is generally measured, a doubling of intensity is equivalent to a 3 dB increase (79).

However, the relationship between the intensity and duration of noise and the resulting hearing loss is not straightforward. For a particular stimulus there is an intensity below which no damage occurs regardless of the duration of the noise. There is also an intensity above which acute damage to the cochlea and severe hearing loss is immediately obvious and more severe than would be predicted

from the equal energy hypothesis such as impulse noises, which involve a sharp short very high intensity of sound. In the area between these two critical intensities as the intensity increases more severe cochlear damage and hearing loss is seen (80).

Permanent threshold shift

Effects of noise on cochlear morphology

The main pathology behind PTS is sensory cell death (10). OHCs are more susceptible to damage than IHCs regardless of the severity of the noise (81, 82). Furthermore, basal OHCs are more vulnerable than apical ones (80). This injury can begin during the noise exposure and can continue after the exposure has ended (83, 84). As the severity of noise increases there can be loss of IHCs and of supporting cells (10).

In addition to cell loss other types of injury include stereocilia alterations. These are one of the first manifestations of noise exposure: they have been noted after only 2 minutes of an intense noise exposure, before damage to the hair cell body occurs (85). Types of stereocilia injury include various forms of disarray and floppiness of the individual stereocilia and of the stereocilia bundles and the links that join the stereocilia together. In severe cases there can be loss of stereocilia (85-87).

Swelling and loss of the Type I afferent fibres have also been found following noise exposure (88-91). The swelling and loss of afferent fibres can be followed by eventual loss of the spiral ganglion cells over long time frames of up to two years, even when there has been no loss of hair cells (90, 91). There can also be acute swelling of the stria vascularis and associated loss of the intermediate cells which have been shown to not recover, this can lead to a reduction in the size of the stria vascularis (10).

Mechanisms of damage

During physiological levels of noise cochlear energy needs increase to maintain cochlear amplification and transduction processes, this is met through increased blood flow and oxygenation (92-94). However, very high or sustained levels of noise result in a combination of mechanical and metabolic injury to the cochlea, with both types of injury leading to initiation of apoptotic or necrotic cell death pathways (95). The mechanical pathway of damage results from excessive vibration of the basilar membrane and organ of Corti which leads to immediate physical damage of cochlear structures such as rupture of Reissner's membrane, disruption of the reticular lamina and separation of stereocilia from the tectorial membrane (80). Whether subsequent to mechanical damage or independently, metabolic pathways of injury can be activated and these are related to the noise induced over-activity of the hair cells (96).

Central to the metabolic pathway is the generation of reactive oxygen species (ROS) (97). ROS include oxygen based free radicals and molecules which are not free radicals but easily generate free radicals such as hydrogen peroxide. Free radicals are molecules that have an unpaired molecule. ROS include superoxide, hydroxyl radical and peroxynitrate as well as hydrogen peroxide (97). During normal function ROS have important functions in the cochlea as signalling molecules (98), during high levels of noise they act to destabilise other molecules and break down molecular interactions and cause damage to cellular proteins, lipids and DNA (97). The cochlea has endogenous antioxidant defence mechanisms to protect against ROS damage, but these processes are overwhelmed following high levels of noise (97).

ROS have been shown to be generated in the cochlea by overdriven mitochondria (99), excitotoxicity (99) and ischaemia/reperfusion injury (Figure 8). Glutamate excitotoxicity occurs when the neurotransmitter glutamate is released in excessive amounts during excessive noise exposure (88). During high levels of noise exposure the IHCs release large amounts of the neurotransmitter glutamate into the synapses with the type I afferent fibres. Prolonged depolarisation of the post synaptic glutamate receptors leads to a large influx of ions (Ca^{2+} and K^{+}) and

passive entry of Cl^-. This can cause water to move into the afferent dendrites causing swelling and rupture (88).

When blood flow is insufficient to meet the increased energy needs following noise exposure, cells are deprived of oxygen which leads to over-driven mitochondria and increased leakage of superoxide (97). The presence of ROS can lead to the peroxidation of lipids which can result in the formation of harmful products such as the prostaglandin, 8-isoprostane-F2α, a vasoconstrictor. This causes vasoconstriction and reduced blood flow leading to further ROS production and decreased blood flow so that a damaging loop occurs. (100, 101). Reperfusion, which occurs when blood flow returns, also generates ROS. Reperfusion means that there is a greater supply of oxygen which can be converted to superoxide or react with existing superoxide (97).

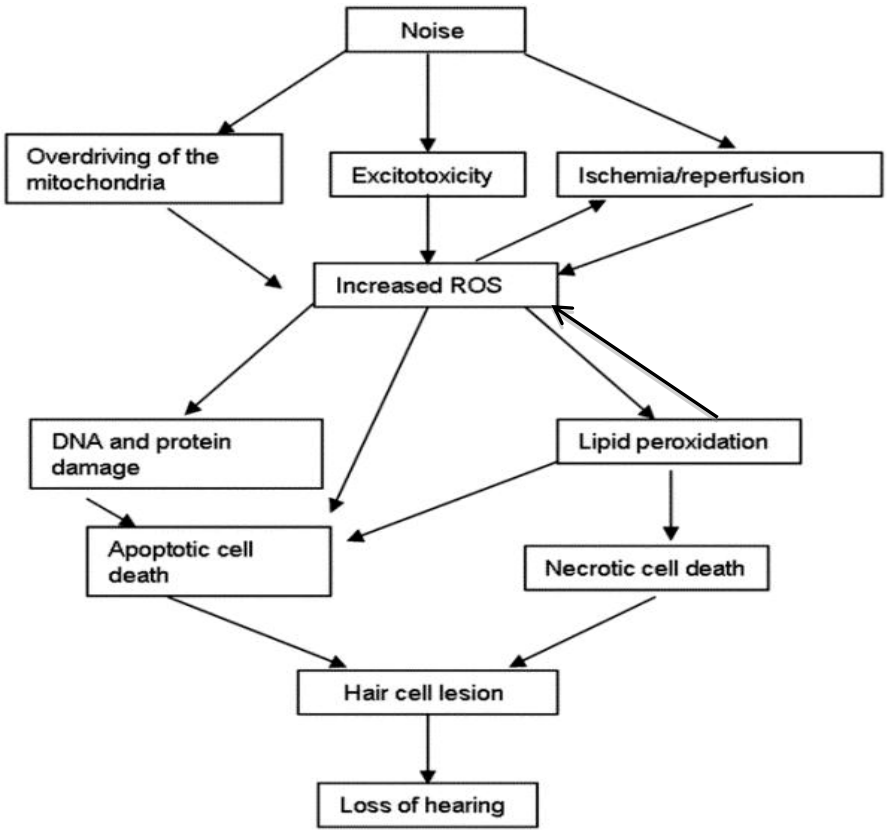


Figure 8: Potential pathways of NIHL. Adapted from (97).

The products of ROS induced cellular damage can initiate cell death through

apoptosis or necrosis. Apoptosis is considered an active cell death pathway in which damaged cells can be removed in an orderly manner to limit the lesion spreading to other cells. Necrosis is a passive type of cell death in which cells rupture and an inflammatory response occurs in other cells which spreads damage to remaining cells (98). It is unclear what factors lead to the initiation of one type of cell death over another. Both types are present immediately (95, 102) and in the post exposure period (84, 102), following both low and extremely high noise exposure levels (95, 102). However, apoptosis is the major cell death pathway involved in the post exposure expansion of the cochlear lesion. This is illustrated by the presence of many more apoptotic than necrotic nuclei in the expansion area (102).

Cell death and survival pathways and their interaction are still being defined, but it is clear they do not operate in isolation from each other (97, 98). Both the intrinsic apoptotic pathway, which is associated with intrinsic stressors such as ROS production, and the extrinsic (outside the cell) pathway, which is associated with external stressors such as the breaking of cellular junctions, are activated in the noise damaged cochlea (95, 103-105).

The cell death pathways are initiated and executed by caspases, a family of enzymes that degrade proteins (106). Operating upstream of caspases are the Bcl-2 family of proteins. This family includes both anti-apoptotic members such as Bcl-2 and Bcl-xL and pro-apoptotic members such as Bax and Bak. The balance of the pro and anti-apoptotic members can determine the fate of cells after injury (107), for example, the pro-apoptotic gene Bak has been found to be highly expressed following a noise exposure that caused PTS and hair cell loss whereas the anti-apoptotic BcL-xL was expressed following a TTS exposure that caused no hair cell loss (107).

Temporary threshold shift

TTS can range from a small change in threshold with recovery to baseline levels within minutes or hours, to very large but fully reversible shifts which recover

over weeks. There is also generally a TTS component to PTSs which recovers over time, leaving only the PTS component.

While PTS is strongly associated with hair cell death there is little or no hair cell death associated with TTS (9, 10). One of the main types of injury that has been associated with TTS is excitotoxic injury, with the presence of type I afferent terminal swelling and IHC vacuolisation in the area of afferent terminal swelling (10, 90, 91, 108). The swelling and loss of afferent fibres can in the case of large TTS's be followed by eventual loss of the spiral ganglion cells over long time frames of up to two years, even when there has been no loss of hair cells or change in thresholds (90, 91).

Changes in the architecture of the cochlea such as buckling of the pillar cells, collapse of the space of Nuel and contraction of the organ of Corti have also been strongly associated with TTS (9, 10, 109). A fully reversible buckling of the pillar cells has been found after a TTS of around 40 dB in the chinchilla. This resulted in a decrease in height of 5-7 μm resulting in a decrease in the height of the organ of Corti. This buckling was associated with uncoupling of the stereocilia from the tectorial membrane in the TTS region, whilst they remained attached to the OHC body. This decoupling could act to attenuate hair cell stimulation thus providing a protective mechanism. (9). Other investigators have found no buckling of pillar cells at any intensity (109) or only in response to very high intensity exposures associated with PTS in the CBA/CaJ mouse (10).

Collapse of the outer space of Nuel has been found following a fully reversible TTS of around 50 dB in the CBA/CaJ mouse. This was associated with a buckled arching portion of the Hensen's cells and a decrease in the organ of Corti. Recovery of the space of Nuel and of Hensen's cell height has been seen by two weeks post-exposure (10).

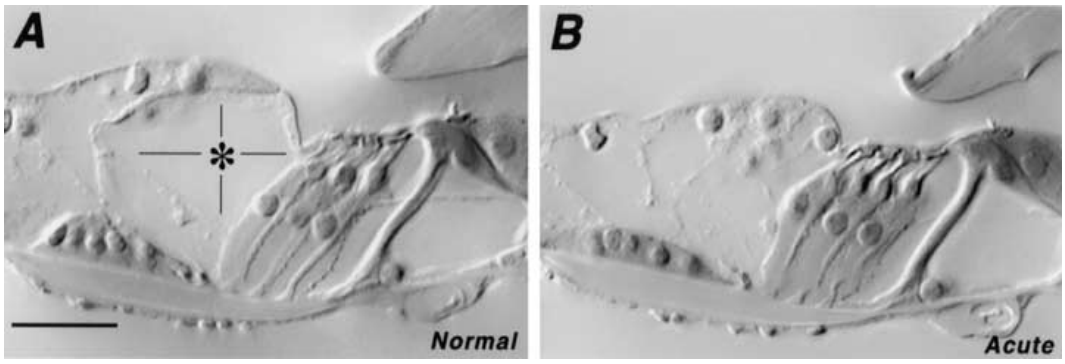


Figure 9: A – Control image from the CBA/CaJ mouse showing the outer space of Nuel (illustrated by asterisks). B – reversible collapse of the outer space of Nuel 24 hours after a large TTS near the threshold of reversibility (~ 50 dB). From (10).

Additionally, a reversible contraction of the organ of Corti has been found in response to low level noise exposure in an in-vitro preparation of the guinea pig temporal bone. After noise exposure Hensen's cells collapsed towards the tunnel of Corti around a hinge point between the third row of Deiter's cells and the third row of OHCs (Figure 10) (109). These changes in cochlear architecture may lead to changes in cochlear micromechanics and sensitivity and may serve as a protective mechanism, reducing the sensitivity of the cochlea to further stimulation (9, 10, 109).

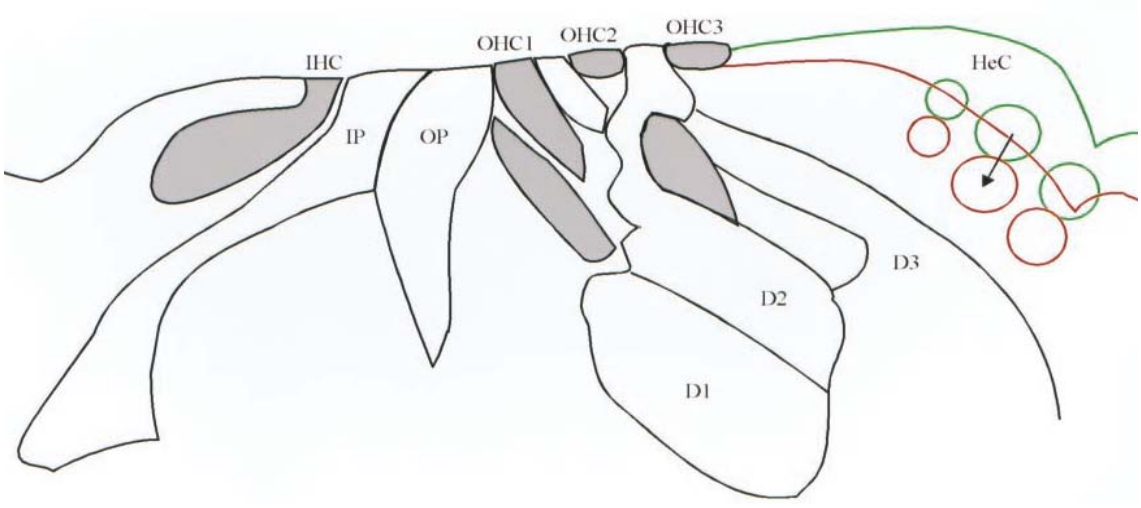


Figure 10: Representation of the contraction of the organ of Corti in response to noise exposure. The circles represent the Hensen's cells lipid droplets. The green circles represent the position of the Hensen's cells before noise exposure. The red circles represent their position after noise exposure. HeC, Hensen's cells. OHC1-OHC3, D1-D3, Deiter's cells rows 1-3 (109).

There is emerging evidence that the small amounts of TTS that are generated in response to low levels of elevated noise are a reflection of an adaptive response to the increased noise rather than a reflection of injury (110). P2RX2 knockout mice which lack the gene which encodes for the $P2X_2$ receptor that assembles ATP gated

ion channels, do not have TTS following low-level noise exposures which cause small amounts of TTS in white mice (around 15 dB half an hour post-exposure). In contrast, knockout mice were more susceptible to PTS than white mice (110). This suggests that TTS following low levels of elevated noise reflects a cochlear protective mechanism which is driven by the release of ATP from $P2X_2$ receptors into the endolymph of the scala media. This has been shown to reduce the EP which reduces transduction and synaptic transmission reducing cochlear sensitivity (110, 111). This reduced activity during noise may protect the cochlea against damage created by such things as glutamate excitotoxicity and ROS activation. In the absence of this adaptive response to noise there is increased PTS (110). Additionally, humans with a history of noise exposure and a genetic mutation of the P2RX2 gene and which consequently may lack this adaptive response have increased susceptibility to high frequency hearing loss (112).

There is also evidence that TTSs which are near the threshold of reversibility are more damaging to the auditory system than previously thought (90, 91).
They have been associated with non-reversible damage to pre and post-synaptic areas and subsequent slow degeneration of the spiral ganglion cells despite recovery of thresholds, suggesting that severe TTS may be more damaging to the auditory system than previously thought (90, 91). TTS of around 40 dB 24 hours after noise exposure was associated with damage to pre-synaptic ribbons and loss of afferent terminals. There was no recovery of these synaptic areas despite full recovery of ABR and CAP thresholds and DPOAEs within 2 weeks of the exposure. There was also reduction in the amplitude of supra-threshold auditory brainstem ABR responses (ABR) despite the return of thresholds to normal levels suggesting the loss of spiral ganglion cells. This was followed by slow loss of the spiral ganglion cells: two years post-exposure around 50% were missing in the area corresponding to the area of synaptic degradation (90). In contrast in those areas in which threshold shift was smaller at 24 hours (around 20 dB) there were minimal synaptic changes, no supra-threshold reduction in ABR amplitude and no slow loss of spiral ganglion cells (90).

Sex differences in susceptibility to NIHL

This section relates to question 1 of Becker et al's (24) set of experimental questions for the investigation of the origin of sex differences in susceptibility (Figure 1), which involves first establishing whether there is a difference between males and females in susceptibility to NIHL. This section will analyse the range of evidence for the presence of a sex difference in susceptibility. Some evidence suggests a difference in susceptibility to NIHL in males and females. The evidence for this comes from multiple sources including analysis of threshold shifts sustained by those exposed to long-term industrial noise (113, 114) as well as comparison of threshold shifts following controlled noise exposures, in both humans (1) and research animals (2, 115). In contrast, other evidence shows no apparent sex difference (27, 116, 117).

Some studies that have analysed permanent threshold shifts sustained by those exposed to long-term industrial noise suggest that there is a sex difference in susceptibility, with females less susceptible to NIHL (113, 114). For example, females sustained around 15 dB less threshold shift at 4 kHz following 10 years of industrial noise exposure of around 89 dB L_{Aeq} (113). This data was corrected with aging curves obtained from a local control group, however, neither the control group nor the noise-exposure workers were screened for non-occupational noise exposure and there can be big differences between males and females in their non-occupational noise exposure exposures, with males having greater exposure levels (17-22).

Others have found no apparent difference in susceptibility. A study of industrial textile workers estimated workers cumulative noise exposure over their working life and predicted their threshold shift based on their noise exposure. The difference between the actual threshold shifts sustained and the predicted threshold shift was calculated. Those with more threshold shift than predicted were considered a susceptible group and those with less than predicted were a resistant group. There were no significant differences in the number of males and females in the susceptible and resistant groups (116). A retrospective analysis of audiometric data also found no differences in susceptibility. This study involved

audiological clinic attendees with a self reported history of significant occupational noise exposure. Thresholds were adjusted using age and gender corrections and no significant differences were found between males and females (117). However actual noise level measurements were not available in this study which makes it difficult to draw firm conclusions about susceptibility differences.

Evidence suggesting a difference in susceptibility also comes from studies that have compared threshold shifts following controlled noise exposures. Some of these studies exposed their participants to noise that is greater than what is considered to be an acceptable maximum daily exposure (100% dose) in New Zealand. This is based on the occupational safe noise exposure limit of an $L_{Aeq,8h}$ of 85 dB as stated in Regulation 11 of the Health and Safety in Employment Regulations (1995) (118). These regulations utilise a 3 dB exchange rate in which a 3 dB increase, which is equivalent to a doubling of intensity, results in a reduction in the allowable exposure time by half (79). For example, using these regulations the Ward study (1) which utilised an exposure of 116 dB SPL at 2800 Hz for 3 minutes had a noise dose of 625% (119). Another very recent study which utilised a digitised music exposure of 100 dB L_{Aeq} for 4 hours (27) had a noise dose of 500% (119). However the noise exposure in this American study did not exceed a 100% noise dose using the American Occupational Safety and Health Administration (OSHA) standards.

While this safe noise exposure limit has been developed based upon a statistical analysis of risk to limit harm over the length of an individuals working life and is not meant to reflect the risk of damage from a single exposure (120), it is possible that some harm may have occurred to some individual's auditory systems in these situations, even if thresholds returned to normal (90, 91).

The most thorough investigation of the topic of sex differences in TTS in humans is that of Ward who investigated the subject in young adults (1). He found that following a low frequency pure-tone noise exposure (700 Hz, 5 minutes, 125 dB SPL) females had significantly less TTS than males: 14.3 dB and 21.45 respectively, standard deviation (SD) 10 dB. Following a high frequency pure-tone noise

exposure (2800 Hz, 3 minutes, 116 dB SPL), females had significantly more TTS than males: 25.9 and 20.46 respectively (SD 10 dB) (1). Data from the chinchilla also shows a sex difference in susceptibility to NIHL. Females had less PTS and TTS in the low frequencies, and more PTS and TTS in the high frequencies, following a 150 dB SPL impulse noise exposure. However, male chinchillas had slightly greater OHC loss and substantially greater IHC loss at all frequencies (2). There were also sex differences in TTS in response to a 500 Hz 95 dB SPL continuous noise, with female chinchillas showing more threshold shift than males (115). When assessing sex differences in the chinchilla it should be noted that female chinchillas tend to be larger than male chinchillas (2) whereas human females tend to be smaller than male humans (121).

Differences have also been found in recovery from TTS following a twenty minute, 105 dB L_{Aeq} pink noise exposure with females showing a greater recovery rate than males, with recovery measured over 60 minutes (122). Another study found no differences in either initial TTS or in recovery from TTS following a 100 dB SPL 1.2-2.4 kHz, 1 hour, interrupted exposure, with recovery measured over 90 minutes (123).

Evidence suggests that presence and size of differences in susceptibility may differ according to the intensity of the noise exposure. A comparison was made between a population of male and female industrial workers with low level (83 dB L_{Aeq}) noise exposures and a group with a high level (98 dB L_{Aeq}) exposures. Females in the high intensity (98 dB L_{Aeq}) exposure group had significantly smaller threshold shifts than males. There were no significant differences between males and females in the low intensity (83 dB L_{Aeq}) exposure group. This data showed large differences in threshold shifts between males in the 83 and 98 dB L_{Aeq} exposure groups, but not between females in the 83 and 98 dB L_{Aeq} groups, showing that females were equally vulnerable to high and low intensity noise exposures whereas males were more vulnerable to higher intensity exposures (114). This suggests the possibility of a protective factor which operates in females and not males at high intensities but not at lower ones.

It has also been found that the sex difference in 4 kHz TTS following a 2800 Hz pure tone exposure (3 minutes, 116 dB SPL) is greater than that obtained following a 1400-2800 Hz broadband noise exposure (3 minutes, 116 dB SPL). Following the pure tone exposure there was a 30% difference in TTS with females having a greater shift than males (25.9 for females and 20.46 for males), whereas following the broadband exposure there was no difference in TTS (1). As less energy reached the 4 kHz cochlear region during the broadband noise exposure as indicated by the lesser amount of 4 kHz TTS (14.2 dB following the broadband noise exposure and 25.9 dB following the pure tone exposure) this also suggests that the differences in susceptibility may be related to the intensity of the noise exposure with less difference in susceptibility following less intense noise exposures (1, 114). A recent study which used a 100 dB L_{Aeq} digitised music exposure (4 hours), found only a small threshold shift at 15 minutes post-exposure (6.3 dB ± 3.9 SD) and also found no sex differences in threshold shift (27).

Summary and conclusion

The above evidence suggests the possibility of a difference in susceptibility to NIHL between males and females. A sex difference in susceptibility has been shown for both TTS in humans (1) and animals (2, 115) and for PTS in humans (113, 114) and animals (2). The evidence for differences in recovery from threshold shift is very mixed with both studies having different outcomes (122, 123).

However, there are differences in outcomes in those studies that have looked at differences in susceptibility following long-term occupational noise exposure, with some studies suggesting a difference (113, 114) while others suggest that there is no difference (116, 117), although one of these studies had no measure of the relative noise exposures of their male and female participants (117). In all studies of that nature there is the important issue of a lack of control over the total noise exposures obtained by the participants. Even in those studies where the workplace noise exposures were carefully measured (113, 114, 116), there is a lack of control over the non-occupational noise exposure of the participants. When looking at the issue of sex differences in susceptibility to NIHL this is important as there are a

number of differences in non-occupational noise exposures between the sexes (17-22). This could lead to the concern that apparent differences in thresholds are not a true reflection of a difference in susceptibility but are due to differences in the noise exposure. Despite these concerns, this is important evidence to consider as it reflects potential differences in PTS in humans whereas those studies that have been able to have tighter control over the noise exposures have looked at TTS.

Other differences in outcomes between studies may reflect the intensity differences as discussed above (1, 114). Evidence suggesting that the size or presence of sex differences in susceptibility to NIHL may vary according to the magnitude of the noise exposure has been shown in humans for both PTS (114) and TTS (1, 27). The PTS study compared sex differences in susceptibility following exposures to different intensities of a similar noise (114) whereas the TTS comparisons have been between noise exposures with different frequency characteristics, for example, a 2800 Hz pure tone exposure (3 minutes, 116 dB SPL) and a 1400-2800 Hz broadband noise exposure (3 minutes, 116 dB SPL) (1). Given that pure tone exposures are considered more damaging than an equivalent energy broadband exposure (124), it would be interesting to compare two exposures of similar frequency characteristics but different intensities to clarify the effect of intensity on sex differences in susceptibility to NIHL.

Other explanations could be that although the output of the noise exposure generating device was the same for each participant, the amount of noise that reached the cochlea may have differed between males and females due to differences in outer ear characteristics. For example, ear canal volume is smaller in females (125-128). Variations in the characteristics of the ear canal and ear drum have been demonstrated to lead to large individual variations in the SPL measured at the ear drum for a particular audiometer dBHL output (129, 130). For example, individual values of measured SPL near the ear drum for a given output have been shown to vary by as much as 20 dB in adults at 3 kHz (130). Those with smaller ear canal volumes will have a greater SPL as measured at the ear drum. This may obscure real differences in susceptibility that would be apparent if the noise

exposure reaching the cochlea was equal. These factors will be discussed in more detail later.

Becker et al's (24) set of experimental questions for the investigation of the origin of sex differences in susceptibility suggest first establishing whether there is a difference between males and females in susceptibility to NIHL. The evidence discussed in this section does not allow a conclusive declaration that there are sex differences in susceptibility to NIHL. There are inconsistencies in the data that may be related to uncertainties about the equality of the total noise exposure (occupational and non-occupational) obtained by the participants, the effect of the magnitude of the noise exposure and concerns that the amount of noise that reached the cochlea may have differed between males and females due to differences in outer ear characteristics, even when the output of the noise exposure generating device was the same for each participant. There is also no clear picture of whether there are sex differences in recovery from TTS.

Given these factors, the first aim of this thesis was to determine whether males and females differed in TTS and TEOAE shift and recovery from TTS and TEOAE shift after exposure to a 3kHz pure-tone, 100 dB L_{Aeq}, 15 minute, noise exposure. This allowed comparison of TTS and TEOAE shift in a group of individuals who had been screened to ensure they had low levels of occupational and non-occupational noise exposure. Additionally, an individualised dBHL to real-ear SPL (reSPL) transform was used to ensure that each participant received a 100 dB L_{Aeq} exposure at the ear drum. This ensured that the noise exposure reaching the cochlea in males and females was as equal as possible so that real differences in susceptibility to noise exposure were not obscured. Furthermore, as this exposure was of similar frequency but lower amplitude than that used by Ward (1), this allowed direct comparison with these results to determine whether the magnitude of the exposure does indeed impact on the presence and size of sex differences in susceptibility to NIHL.

The role of activational effects of estrogen and progesterone on susceptibility to NIHL.

An explanation for the lack of a sex difference found in some studies (1, 27) could be that there is no sex difference. However, Becker et al (24) suggest one must consider the possibility of sex effects working in the opposite direction to cancel each other out before determining that there are no sex differences. The studies above have not done this and share in common a lack of reference to the hormonal status of the female participants and the important effect that this may have on the presence of sex differences in susceptibility. For example, if a difference in susceptibility is small and variable, apparent during some stages of the menstrual cycle and not at others, then a sex difference could be easily missed, or its size underestimated if most participants happen to be measured during a phase when the sex difference is very small or absent (24). Becker points out that this can be especially problematic for studies with small sample sizes such as the studies above (N ranged from 8–24 M and 12-25 F) (1, 27, 122, 123). There is no information provided in the studies discussed on which to base any assumptions about the hormonal status of the female participants.

This section relates to question 2 of Becker et al's (24) set of experimental questions for the for investigation of the origin of sex differences in susceptibility (Figure 1), which involves determining whether sex differences in susceptibility are related to the effects of sex hormones at the time of testing. This section discusses in detail the evidence suggesting the importance of circulating levels of the female sex hormones estrogen and progesterone on sex differences in susceptibility to NIHL.

As the presence of a hormone receptor in a cell is considered presumptive of an action of the hormone on that cell type (53), the presence of estrogen receptors in the cochlea (7, 131, 132) suggests that estrogen has an important role in the functioning of the cochlea. In contrast, to date no progesterone receptors have been identified in the cochlea (133), although there are also no reports of their absence. It may be that there has been no research interest in establishing their presence or perhaps researchers have looked for them and not published their

absence. However, there is some unpublished data from a thesis which indicates that progesterone receptors may be present in the spiral ganglion cells of human foetal tissue (134). This suggests that more studies with a range of different antibodies are required before determining the presence or absence of progesterone receptors in the cochlea (134).

The presence of both ER α and β has been established in rats, mice (7, 131) and in humans (132). In mice and rats both receptor types were demonstrated in the nuclei of IHCs and OHCs as well as in supporting cells, however with no ERβ found in the OHCs of mice (131). They have also been found in cells of the spiral ganglion, Reissners membrane, stria vascularis and in blood vessels (7, 131). Other data does show the presence of ERβ in both the OHCs and IHCs of white mice as well as the spiral ganglion neurons. There were no differences in expression between male and female mice (7). In human adults ERα was found in the spiral ganglion and ERβ in the stria vascularis. In human foetal tissue ERα was stained lightly in the spiral ganglion and ERβ was not found at all (132). While there may be differences in particulars of what receptor type is found where amongst the different studies, it is clear that ER's are present in the cochlea and have been found in cell types that are involved cochlear amplification, transduction, maintenance of the EP and cochlear blood flow as well as in supporting structures.

There are few studies that have investigated the topic of the effect of circulating hormones on NIHL in humans. Those that have, have used menstrual cycle stage as a marker of hormone status rather than direct measurement of hormone levels and have looked at TTS. While one study has shown no cyclical differences in TTS with no significant differences during different menstrual cycle stages (122) others suggest that circulating levels of sex hormones do modulate TTS with a trend for there to be less TTS during the early-follicular phase of the cycle when both progesterone and estrogen levels are low (3, 4).

Swanson & Dengerink (3) examined the fluctuation in TTS at 4 kHz and 6 kHz following a white noise exposure during the early-follicular, late-follicular and luteal phase and also measured TTS in males and in females using oral

contraception (OC) at equivalent intervals. For menstrually cycling (MC) females there were cyclic variations in TTS which did not occur in males and OC females, whose TTS was similar at each interval. MC female TTS was smallest during the early-follicular phase when both progesterone and estrogen levels are low and larger during the late-follicular phase when estrogen levels are very high and the luteal phase when progesterone levels are very high. This suggests that low estrogen and progesterone levels are associated with low levels of TTS (3).

During the early-follicular phase female TTS was less than male and OC female TTS. This trend occurred at both 4 kHz and 6 kHz and was significant at 6 kHz (MC = 5.8 dB, OC = 13.5 dB and male = 11.4 dB). During the late-follicular and luteal phases there were no differences in TTS. (3). Importantly, in another study (135) when MC females were not classified according to hormonal levels there was no apparent sex difference in susceptibility to TTS following the exact same level and intensity and duration of white noise exposure (110 dB SPL, 5 minutes) (135). This indicates the importance of accounting for hormonal status when investigating sex differences in susceptibility to NIHL as the presence of a sex difference in susceptibility to this type of noise as shown by the presence of an activational hormonal effect on TTS was obscured when treating females as a homogeneous group.

Oral contraception use has also been found to impact on TTS. It has been found that OC females had statistically more TTS at 4 kHz than males and MC females (15.4 compared with 9.3 and 8.8 dB respectively) (135). Other research has also found that females using OC have more TTS (122).

There is no data comparing the threshold shifts following controlled noise exposures in post-menopausal females in whom the levels of circulating hormones have naturally declined and young females and males. Interestingly, in the post-menopausal (near the end of lifespan) Mongolian gerbil no sex differences in NIHL were found following a 3.5 kHz pure-tone, 113 dB SPL exposure (136).

The potential importance of estrogen in modulating threshold shift is also demonstrated by data which shows greater NIHL when the cochlea is treated with an anti-estrogenic compound before noise exposure in the male Mongolian gerbil (5). This is also shown by evidence showing that estrogen treatment increases protection against threshold shift in the chinchilla, with higher doses showing more protection (6). Other research shows that removal of ERβ worsens noise induced threshold shifts (7). In contrast, while there is little data looking at the effect of progesterone on NIHL a preliminary study looking at the effects of progesterone pre-treatment in the chinchilla showed that there was either no effect on threshold shifts or perhaps a small worsening in noise induced threshold shift (6).

Tamoxifen is an anti-estrogenic compound which acts on ERs and inhibits the genomic action of estrogen (5, 137). Male Mongolian gerbils that were administered Tamoxifen five hours prior to noise exposure (8 kHz NBN, 30 minutes, 108 dB SPL), had greater PTS 30-35 days post noise-exposure in the 1-7 kHz and 8–15 kHz frequency bands. They also had worse DPOAE amplitudes in the 1-7 kHz band. This suggests that the presence of estrogen protected against threshold shift in cochlear regions that received high amounts of energy from the noise exposure, as well as in regions which received less energy as they were apical to the noise exposure frequency band. However, estrogen protected against DPOAE shift only in those regions of the cochlea that received lesser amounts of energy (5).

17-β estradiol pre-treatment has been shown to have a protective effect against both impulse noise and continuous noise in the chinchilla (6). Two weeks of pre-exposure treatment with estrogen protected against high frequency threshold shift following an impulse noise exposure. Additionally, there was some significant protection against basal OHC loss (6). Further experiments were performed looking at the effect of either 3 or 7 days of pre-exposure estrogen treatment at either 100μg or 725μg total dose. These treatments also showed protection against high frequency threshold shift following a 4 kHz octave band noise, 105 dB SPL, 4 hour long exposure. The most effective dose was the 7 day, 725μg dose followed by

the 7 day 100μg dose. Despite the protective effect against PTS there was no difference between the groups in either OHC or IHC loss (6).

Further evidence for a protective effect of estrogen is shown by the more severe NIHL demonstrated in ERβ knockout mice (7). Comparison was made of the threshold shift between ERα knockout mice, ERβ knockout mice and aromatase knockout mice following a 6-12 kHz BBN, 45 minutes 100 dB SPL exposure. ERβ and aromatase knockout mice showed significantly more TTS than white mice, indicating that ERβ has a role in protection of the cochlea following noise exposure. Additionally, an ERβ agonist protected against threshold shifts in white mice and aromatase knockout mice. There was no difference in TTS between ERα knockout mice and white mice indicating that ERα is not involved in protection from noise exposure. There was no difference between males and females in the threshold shifts of white mice or the knockout mice following noise exposure (7).

These findings seem to be in contrast to the human data discussed above (3, 4), which indicates that menstrually-cycling females had less TTS in the early-follicular phase when estrogen levels are at their lowest. Some evidence that female sex hormones effect have a negative effect on NIHL has been demonstrated in the C57BL/6J (B6) mouse. Male and female B6 mice have similar hearing until around three months (young adult) and then hearing loss accelerates more rapidly in the female. Intact female B6 mice had more hair cell loss following low level extended nightly noise exposures than gonadectomised mice: after low frequency exposures they had greater loss of apical IHCs than gonadectomised females and after high frequency exposures they had more basal OHC and IHC loss. The difference in hair cell loss between intact and gonadectomised females was greater than the difference in threshold shifts (8).

The mechanisms by which estrogen and progesterone affect susceptibility to NIHL

There are many pathways through which sex hormones could affect NIHL. In the central nervous system (CNS), estrogen has been shown to have wide-ranging effects including modulation and protection of neuronal activity, acetylcholine synthesis, direct antioxidant effects and has been shown to involved in reduction

of lipid peroxidation and apoptotic cell death (138). Progesterone has also been shown to have a neuroprotective role in the CNS including reduction of glutamate induced oxidative injury (139). There are both reports suggesting that progesterone does and does not interfere with the beneficial effects of estrogen (139).

In the cochlea estrogen and/or progesterone have been shown to act on pathways that are associated with NIHL such as cochlear blood flow (73, 140, 141) and apoptotic cell death (142). There is also data suggesting that estrogen and progesterone don't exert their effects on NIHL by acting directly acting on hair cells (5, 6, 137). Additionally, it remains unclear whether estrogen and progesterone are acting through genomic or non-genomic pathways (6), via direct effects or via interaction with other factors (7).

Progesterone has been shown to alter blood pressure and cochlear blood flow in the rat. It was shown to enhance the increase in blood pressure that was caused by a vasoconstrictive agent and to reduce the elevation in cochlear blood flow (141). Estrogen and progesterone have also been shown to effect the cochlear response to vasoconstrictive compounds in the guinea pig. Progesterone enhanced the increase in blood flow that occurred after treatment with the vasoconstrictive compounds. Estrogen treated animals showed less increase in blood pressure and cochlear blood flow than non-treated animals (140).

Estrogen, via its inhibition of rho-kinase in the spiral modiolar artery (SMA), may explain why females did not show the enhanced myogenic tone (sustained vasoconstriction) of the SMA in the presence of a nitric oxide synthesis (NOS) blocker that males did (73). Rho-kinase is a kinase which has an important role in regulating cellular morphology and movement (143). This sex difference in the vasoconstrictive response was found to be related to differences in the calcium sensitivity of the SMA (73). This difference in calcium sensitivity in the SMA following inhibition of NOS was found to be regulated by rho-kinase, which increases calcium sensitivity, thus increasing sustained vasoconstriction. Females had lower basal rho-kinase levels in the SMA than males. NO inhibited rho-kinase

activity so when NOS was inhibited rho-kinase increased, increasing calcium sensitivity and vasoconstriction. As females have lower basal rho-kinase levels they were less sensitive to the effects of NOS inhibition (73).

Estrogen has also been shown to be involved in apoptotic cell death pathways after insult to the OHCs by ototoxic substances. Application of 17β estradiol to basal OHC explants has been shown to partially protect against OHC loss caused by gentamicin ototoxicity in a dose dependant manner. This is thought to have resulted from significantly decreased JNK activation in estrogen treated OHCs and significantly less apoptotic cells as determined by reduced TUNEL staining (142).

Various studies suggest that there is a dissociation between the effect of estrogen on thresholds or measures of whole cochlear output such as the CAP and the effect on either OAEs or measures of hair cell loss. For example, tamoxifen worsens CAP thresholds after noise in the 8-15 kHz band but doesn't significantly worsen DPOAEs (5). This suggests that estrogen is protecting against threshold shifts more than OAE shifts and that its role in protecting against NIHL may not be operating through the OHCs. This is also shown after treatment with 17β estradiol where despite a protective effect of estrogen against PTS there was no difference in either OHC or IHC loss (6). The lack of difference in hair cell loss between the estrogen treated and control animals could suggest that estrogen is again exerting its protective effect through other mechanisms such as modulating the function of stria vascularis structures rather than a direct effect on the hair cells (6). In fact, estrogen has been shown to effect stria vascularis marginal cell function: application of 17β estradiol has an inhibitory effect on strial marginal cell function in the gerbil. 17β estradiol reduces secretion of potassium by inhibiting through non-genomic mechanisms the function of the I_{ks} channels, an ion channel in the apical membrane of the marginal cells, which are important in the secretion of potassium (137). However this effect only occurs at high concentrations that only occur in vivo at the end of pregnancy (137), suggesting that the alterations in auditory function (and potential changes in susceptibility to NIHL) that occur over the menstrual cycle may not be modulated by this effect (137). There was no

significant effect of progesterone on marginal cell function at any concentration (137).

There was also a dissociation between the effects of sex hormones on thresholds and hair cell loss following gonadectomy in the B6 mice, but this study found a greater difference in hair cell loss than there was in threshold shifts (8). This study differs from the others in that estrogen seems to be NIHL enhancing in the B6 mouse, whereas it was protective against NIHL in the other studies (5-7).

An investigation has occurred as to whether estrogen acts via its receptors alone to impact on NIHL or whether its effect on NIHL occurs via interaction with brain derived neurotrophic factor (BDNF) (7). BDNF has been found to effect synaptic transmission and neuronal survival in the CNS and has also been found to be sensitive to the effects of estrogen in the CNS (144). This indicated that the increased susceptibility to NIHL due to the absence of ERβ receptors in the cochlea may perhaps be mediated by the low levels of BDNF in the cochleae of ERβ knockout mice relative to white mice and ERα knockout mice. There were no sex differences in the baseline expression of BDNF (7).

After a TTS inducing noise exposure there were sex and strain specific differences in BDNF. Male and female white mice both had large decreases in BDNF, with a much larger decrease in males. ERβ knockout mice also had a reduction in BDNF, but this was smaller than in white mice. Male ERβ knockout mice had a slight decrease in BDNF whereas female ERβ knockouts had no significant change (7). The decrease in BDNF occurred due to its release and degradation; as white mice had higher levels of BDNF it was released in greater amounts than in ERβ knock-out mice to try and protect the cochlea, hence the greater reduction in BDNF. Interestingly, both male white mice and ERβ knockout mice had greater decrease in BDNF, however this was not reflected in any sex difference in TTS after the noise exposure (7).

There is some evidence that estrogen exerts its effects on NIHL through genomic mechanisms (6). Experiments were performed looking at the effect of either 3 or 7

days of pre-exposure estrogen treatment at either 100µg or 725µg total dose on threshold shift following a 4 kHz octave band noise, 105 dB SPL, 4 hour long exposure. The most effective dose was the 7 day, 725µg dose followed by the 7 day 100µg dose. The 3 day pre-treatment was not effective (6). The authors suggest that the effectiveness of the 7 day dosing and relative ineffectiveness of the 3 day dosing protocols suggests that estrogen is exerting its protective effects through the slow acting genomic mechanisms which operate over a slow timeframe rather than a non-genomic pathway which has a shorter timeframe (53).

Summary and conclusion

The human evidence suggests that there may be an activational effect of hormones on TTS (3, 4) which would suggest the presence of a sex difference in susceptibility to NIHL. However, there is some discrepancy in the data with another study showing no cyclical effect (122). However, all of these studies have not directly measured hormonal levels and have estimated hormonal status based on either self-report of menstrual cycle stage (4) or by the measurement of basal body temperature and observation of cervical mucus quality (3). Classifying women into menstrual cycle stages based on indirect criteria such as the above provides only a rough estimate of hormonal status. Self reported menstrual cycle stage is a particularly unreliable method of estimating hormonal status (24) and the other methods of estimating menstrual cycle stage are still imprecise as there are very large inter-individual differences in the measured hormone levels of each menstrual cycle stage (24, 145). Direct measurement of hormone levels is recommended as the best way to establish any relationship between hormone levels and NIHL (24).

Given these factors, the second aim of this study was to determine whether there was an association between estrogen and progesterone levels and TTS and TEOAE shift in female participants and whether males, OC females and females in the early-follicular, late-follicular and luteal phases differed in TTS and TEOAE shift. To the best of my knowledge there has been no investigation of the relationship between measured hormone levels and how this relates to NIHL susceptibility.

Alternative explanations for sex differences in susceptibility to NIHL

This section will briefly discuss alternative explanations to the activational hormonal hypothesis for sex differences in susceptibility to NIHL. This relates to question 3 of Becker et al's (24) set of experimental questions for the investigation of the origin of sex differences in susceptibility (Figure 1), which involves determining if sex differences in susceptibility are related to permanent differentiating effects of sex-hormones during the pre-natal or neo-natal period.

Males and females have been found to differ in the size of many structures of the auditory system which may be the result of these permanent differentiating effects. Differences in these structures may effect sex differences in susceptibility to NIHL.

Organisational effects on auditory structures

There is more complete information about male and females differences in the outer ear than there is on other parts of the auditory system; this is likely due to the easy accessibility of the structures for measurement. The pinna projects from the side of the head to a lesser extent in females (1) and is also shorter in length (1, 146-148). No differences were found in measures of lobe height or width (146). The pinna leads into the opening of the ear canal which is also smaller in females (148). Studies have also consistently found small differences in ear canal length with females having the shorter ear canals (126): 2.2 cm for females and 2.4 cm for males in one study of 3 females and 4 males (149) and 2.4 cm compared with 2.5 cm in a study of 5 males and 5 females (150) although these differences did not reach statistical significance (150) or a statistical test of significance was not performed (149, 150). Ear canal volume is also smaller in females (125) as demonstrated by both physical measurements made in human cadavers (125) as well as in physiological measures of volume (126-128). Data on sex differences in the dimensions and form of the ossicles is mixed with some suggesting no differences (151) and another showing a limited difference: a large study showed a difference in only 4 out of 12 measures of ossicular dimension with females having the smaller dimensions (152). Overall this data shows that the outer ear structures are smaller in females (1, 146-148), which is most likely related to smaller female

body size (121). The data is less clear about sex differences in the middle ear: data on structural differences is more difficult to obtain and mixed (153) and data on middle ear function is also mixed. The size and function of middle ear structures like the outer ear structures is related to body size (154). Differences between studies in the presence of sex differences could be related to body size in the male and female groups differing to a greater or lesser degree in the different studies. No information was given about the relative body size of the male and female groups.

There is some data looking at differences in the function of the middle ear as measured by tympanometry. Some data suggests that females have lower admittance values (128). Other data suggests no difference in admittance values (155). Data from different sized cat species suggests that admittance is greater in larger cats than it is in smaller cat species (154). A study comparing tympanometric values in Caucasian and Chinese people showed no sex difference in admittance for Caucasian people with lower admittance values for Chinese females than Chinese males (127). No sex difference in tympanometric peak pressure values has been found (127, 155). Some evidence also suggests that tympanometric width does not differ between the sexes (127, 155), although this has also been found to be wider in females (128). No sex differences have been found in the resonant frequency of the middle ear as measured by multi-frequency tympanometry (127).

There are sex differences in cochlear length with males having longer cochleae than females. One study found that male cochleae were 14.8% longer: 37.1±1.6 mm long in males and 32.3±1.8 mm in females (156). Another study using pooled data from 11 studies found a smaller difference of 3.36%, with male cochleae being 1.1 mm longer than female (157). In general though cochlear length has large inter-individual variability: in a study of 50 human cochleae, length varied by 35.6% (158).

Data on sex differences in hair cell structure, density or number is sparse, however, shorter cochleae have been shown to have greater hair cell density than

longer ones in the chinchilla (159) and humans (158), although longer cochleae have more hair cells overall (158) suggesting that females might have greater hair cell density and males more hair cells overall. Female cochleae have been found to have slightly higher hair cell density counts than male; however as only 6 out of 44 of the cochleae studied were female these results were inconclusive (160).

The effect on susceptibility to NIHL

Differences in the properties and action of the outer and middle ear lead to differences in the enhancement and transmission of some frequencies of sound. Although there is limited data looking specifically at sex differences in the outer and middle ear and how this relates to NIHL, there is data looking at how the size of these structures relates to NIHL. As the sex differences are mostly size related then this is helpful data to look at.

The anatomy and physiology of the outer ear and middle ear enhances the transmission of some frequencies of sound to the cochlea with an increase in sound pressure level (SPL) at the oval window compared to their SPL in the environment. The exact frequencies and amount of enhancement depend on individual characteristics such as the shape, size, length, and flexibility of the structures as well as head diffraction effects. The increase that the outer ear gives to SPL can be termed the sound transfer function (STF) and at its maximum can be 15-20 dB (126, 161). The smaller volume and shorter length of the female ear canal means that the female STF is shifted towards higher frequencies than the male STF, with females having a bigger STF at 5, 6.3 and 8 kHz. This means that for a noise of a given SPL in the environment that females will have a greater SPL at the eardrum at these frequencies than males. Female STF was significantly lower at 2, 2.5, 12 and 16 kHz meaning females will have a lower SPL at the eardrum at these frequencies than males (126).

The frequency of maximum STF is the frequency at which people have been found to be most susceptible to TTS: when sound energy was focused in the 2 kHz area those who had their maximal STF at 2 kHz had more TTS than those whose

maximal STF was at 4 kHz, presumably as more energy reached the cochlea. When sound energy was focused in the 4 kHz area those who had their maximal STF at 4 kHz had more TTS than those whose maximal STF was at 2 kHz (161). If the energy of real-life noise exposures was dominated by frequencies lower than 2 kHz a STF that was shifted towards lower frequencies as is the case for males may lead to a greater enhancement of these frequencies in males and a higher noise exposure reaching the cochlea (126, 161).

The larger pinna size and greater pinna projection of males may also be associated with greater susceptibility to NIHL: male and female TTS was compared following a soundfield noise exposure (1400-2800 Hz, 15 minutes) and an equivalent noise exposure presented through headphones. There was no difference in male and female TTS following the headphone exposure but males had significantly more TTS following the soundfield exposure. The author suggests that the larger and more projecting pinna of the males may have funnelled more sound into the male ears in the soundfield condition leading to more sound reaching the cochlea and a larger TTS (1).

As with the outer ear there is little data looking specifically at sex differences in the middle ear and how this relates to NIHL, however a link has been suggested between middle ear impedance (resistance to the flow of sound energy) and susceptibility to NIHL suggesting that human middle ear impedance is greater than chinchilla middle ear impedance with humans being less susceptible to NIHL (162). There is some evidence suggesting that males have higher admittance (128), which is the opposite of impedance. This data would suggest that this higher admittance should be associated with greater susceptibility to NIHL in males. Males and females have not been demonstrated to differ in the strength of the acoustic reflex (163), suggesting the acoustic reflex is not associated with sex differences in susceptibility to NIHL.

There is evidence suggesting that sex differences in the size of the cochlea are not associated with differences in NIHL. For example, McFadden et al found differences in the response to noise in the chinchilla but no differences in the length of the

cochlea suggesting that in the chinchilla at least differences in the response to noise are not related to cochlear length (6).

The impact of activational effects of sex hormones on the prediction of susceptibility to NIHL

As part of the process of developing a reliable test of susceptibility to NIHL that could eventually be used in workplaces to detect particularly susceptible individuals, it is important to consider whether sex and circulating levels of female sex hormones are factors that needs to be considered. This is because sex hormones may impact on both susceptibility to NIHL and on the strength of those measures that have been used to predict susceptibility (3, 4, 48-50).

To investigate this, this section will first consider the effect of sex and sex hormones on auditory predictors of NIHL. Much effort has gone into finding predictors of NIHL in the hope of developing a test of NIHL susceptibility. Various measures of auditory physiology have been found to be predictive of NIHL size: these are PTA thresholds and OAE based measures of auditory function including OAE amplitude and efferent suppression amplitude (2-4, 27-34). Sex differences have been documented in these aspects of auditory function (35-47) which evidence suggests may be related to circulating levels of the sex hormones estrogen and progesterone (3, 4, 48-50). Further to this, this section will discuss whether sex hormones act to influence susceptibility to NIHL via their effect on these predictors or whether hormones act independently of these effects to influence susceptibility to NIHL.

This section will begin with a brief introduction to the concept of susceptibility and how an occupational noise management programme might benefit from the measurement of susceptibility.

Background

Individuals differ in the severity and rate of progression and recovery of threshold shift in response to the same noise exposure (25, 164). This difference in susceptibility to NIHL is an important feature of the presentation of NIHL. It has been demonstrated across a range of species following controlled conditions and in humans. For example, in the chinchilla a 161 dB impulse noise exposure led to around 40-60 dB of PTS in some animals whilst others had almost no PTS (164). In

humans 4 kHz threshold shifts have been found to range from ~10 to 70-80 dB in a homogenous group of jute weavers exposed to noise conditions that were relatively stable throughout their work history (25).

The aim of an occupational hearing loss prevention or noise management programme is to prevent individuals from obtaining a NIHL (11). These prevention programmes are based on a determination of NIHL risk that is based on the duration and intensity of the noise. If noise is over a particular duration/intensity combination then NIHL preventative measures must be put in place. In New Zealand this level is an eight-hour continuous A-weighted sound pressure level, $L_{Aeq,8h}$ of 85 dB (165). However, for individuals this risk calculation is complicated by this difference in susceptibility to NIHL. For example, the threshold shifts in the group of jute weavers mentioned above ranged from 10 dB for the least susceptible to 70-80 dB for the most susceptible (25). This is the difference between a barely noticeable and very debilitating hearing loss.

In addition to the noise monitoring and control and audiological monitoring which are essential parts of an effective programme it would be useful to have information about an individual's susceptibility to NIHL. This information could be used to prevent NIHL by accurately establishing individual risk and directing the prevention programme to account for this additional risk by providing such things as extra audiological monitoring, training and hearing protection for those who are particularly susceptible.

Auditory predictors of NIHL

A correlation between baseline PTA amplitudes and TTS has been found in females (3, 4), with those who had higher (worse) thresholds having less TTS. The same trend has also been seen in a combined group of males and females following a 100 dB L_{Aeq} digitised music exposure ($R_2 = 0.1817$, $p < 0.001$) (27) and in a group of male participants following a 105 dB SPL, 10 minute, 2 kHz 1/3 octave band noise (28). Interestingly, data shows that male chinchillas had slightly better low frequency thresholds than females and that females had slightly better high

frequency thresholds than males (as measured by evoked potentials). Following impulse noise exposure female chinchillas had more TTS and PTS than males in the high frequencies (where their thresholds were better) and males had more TTS and PTS than females in the low frequencies (where their thresholds were better) (2).

A positive correlation between baseline OAE amplitudes and PTS has been found amongst naval employees (29), with those who had lower (worse) amplitude OAEs or absent OAEs having more PTS following 9 months of noise exposure on an aircraft carrier (29). The best predictor of a PTS was the TEOAE amplitude in the 4 kHz half octave band, the risk of PTS increased from 3% to 20% as OAE amplitude decreased (29). However, as this study included serving military personnel who had already had significant noise exposure before the study, their low amplitude or absent OAEs could have reflected previous noise damage rather than normal variability in OAE amplitude (29). 4 kHz TEOAEs also predicted threshold shifts from impulse noise exposures: again, those with low amplitude OAEs were more at risk of PTS (30).

It is interesting to note that better auditory function as measured by PTA is associated with more NIHL, while better auditory function as measured by OAE amplitude is associated with less NIHL. This may be related to the fact that the PTA studies were looking at the correlation with TTS, while the OAE study looked at correlation with PTS. This may be a reflection of the different mechanisms underlying TTS and PTS (9, 109, 110). If small amounts of TTS such as occurred in these human studies (maximum of ~13 dB) (3, 4, 27, 28) are thought to be an adaptive response to the increased noise that protects the cochlea from further damage rather than a reflection of injury (9, 109, 110) then perhaps those with good auditory sensitivity have auditory systems which have a greater ability to turn themselves down when stressed by small amounts of noise (reflected in higher amounts of TTS) and hence have less permanent NIHL/better auditory function. This would suggest that when looking at the correlation between small amounts of TTS and measures of auditory function that better PTA and better OAE amplitudes should both be associated with more TTS.

Recent attempts to find a predictor of NIHL susceptibility have focused on efferent suppression magnitude as this has been established as a very effective predictor of NIHL in animals. In the guinea pig there is a negative correlation between efferent suppression strength and PTS, with those who had low efferent suppression strength having higher PTS (31, 32). The correlation is a strong one with a r value of 0.77 ($p < 0.0025$) for the correlation between efferent suppression strength and DPOAE loss (32). Additionally, individual variability in the expression of the α9 acetylcholine receptor subunit was associated with efferent strength and therefore with susceptibility to NIHL (32).

The data investigating the association between efferent strength and susceptibility to NIHL in humans is less clear cut, some investigators have found no correlation between efferent suppression strength and TTS (166, 167) or temporary OAE shift (166). In contrast, one investigator found a positive correlation between efferent suppression and temporary OAE shift (33), instead of the negative correlation found in the guinea pig (31, 32). Additionally, no correlation has been found between efferent strength and PTS following two years of noise exposure in ship engine-room recruits (34).

There are a number of proposed reasons for the difference in the strength of association between efferent suppression and NIHL in humans compared to other species. These include differences in the amount of threshold shift induced: the TTS studies have used lesser levels of noise exposure than used in the animal studies and even the PTS study involved minimal permanent changes, and as the protective effect offered by the efferent system is greater for more severe exposures the efferent system may not have been as active in the human investigations (74). Another important reason may be that in humans, the evidence for the presence of a slow effect is unclear as it has been shown to be minimal at the levels of efferent stimulation which are used in human studies (168). This could be important given that protection from threshold shift may be mediated by the slow efferent effect (169).

Sex differences in the measures of auditory physiology that predict NIHL

Epidemiological studies consistently show that males have worse hearing thresholds than females at all ages particularly in the high frequencies (35-40). Some have found a small difference showing that males have better low frequency thresholds than females (36, 37), while others have found no difference in low frequency thresholds (170). Some studies have found that after adjusting for occupational noise exposure no differences in hearing thresholds were found (117, 171, 172). Interestingly, amongst residents of a non-industrialised and isolated island society there were no differences in hearing between females and males who had not travelled to the mainland for work (172). However, other evidence also suggests that females still have better hearing thresholds when occupational noise differences are statistically controlled for (37, 38, 113, 170, 173). Again, the issue of difference in leisure noise exposures can complicate interpretation of findings.

There are consistent sex differences in OAEs with females having stronger (bigger amplitude) and more numerous OAEs. These differences have been shown in both SOAEs and in evoked OAEs and exist from the neonatal period (41-45). More females than males have present SOAEs and females have more numerous SOAEs (41, 42, 44). Females also have larger amplitude click evoked OAEs such as TEOAEs. This has been shown in human infants (43), children and adults (44, 174) as well as in a number of research animals such as the rhesus monkey (175) and sheep (176). Data showing differences in DPOAEs is mixed with some data showing no difference (177, 178). For example, TEOAEs and DPOAE measured in the same group of young adults showed much larger sex differences in the TEOAE measures, the DPOAE differences were mostly not significant (178), however other studies have found differences in DPOAEs (44).

Sex differences in efferent suppression strength have been found in the CBA mouse (46) and in young human infants (47), although the direction of the difference in unclear. Suppression was found to be larger in the young adult female CBA mice (46), whereas in human infants efferent suppression was greater in males: the

amount of suppression was 3.28 (±0.27) dB in males and 2.32 (± 0.27) dB in females (47).

The role of activational hormonal effects on sex differences in the measures of auditory physiology that predict NIHL

There is considerable data suggesting that estrogen has an important (mostly protective) role in cochlear physiology. There is a less clear picture of the role of progesterone in cochlear physiology with some data suggesting it has no function (49) and other data indicating a detrimental effect (179). However, the data that indicated that progesterone has a detrimental effect examined the role of an exogenous progesterone, a component of hormone replacement therapy, rather than endogenous progesterone.

There are a number of different types of evidence showing an activational role of hormones on auditory physiology. These include differences in auditory function between pre and post-menopausal females as well as differences in function between females using oral contraception and those who are not (menstrually-cycling) as well as differences in function across the menstrual cycle.

Female menopause results in the loss of circulating sex hormones and has been associated with a decline in auditory function. DPOAEs in female CBA mice are maintained at a similar level until early middle age before declining post-menopause whereas male OAE declined steadily through middle age and into old-age (180). Menopause has also been shown to be associated with worse PTA thresholds in human females (181, 182). Female CBA mice who have stronger efferent suppression magnitude than males in youth show a greater decline of efferent strength than males with age, suggesting a protective effect during youth which declines with age (46). In contrast, the removal of circulating hormones through gonadectomy in the female B6 mice caused better low frequency thresholds than occurred in intact female mice, this was associated with less hair cell loss following noise exposure (8).

The effects of oral contraceptive use as reported in the literature are contradictory. Oral contraception has been associated with slightly smaller (worse) amplitude OAEs (183). Others have found OC use to be associated with lower (better) thresholds (48, 122, 184) than either menstrually-cycling (MC) females, regardless of menstrual cycle stage or males (48). OC users did not have the cyclic variation in their thresholds that has been documented in MC females: when OC using females were measured at similar intervals as MC females in the early-follicular, late-follicular and luteal phases their thresholds were stable across the measurement intervals whereas the thresholds of MC females fluctuated, being significantly worse during the early-follicular phase (3).

There is considerable evidence suggesting that auditory function alters over the course of the menstrual cycle, showing an activational effect of hormones on PTA thresholds (3, 4, 48), TEOAE amplitudes (49, 50) as well as efferent suppression magnitude (49).

Some research has found that MC women had their worst thresholds during the first part of the luteal phase (1.34 dB) and their best thresholds during the early-follicular phase (0.34 dB)(48). In contrast others have found a trend towards females having higher PTA thresholds (worse hearing) during the early-follicular phase of the cycle during which both estrogen and progesterone levels are low (3, 4). For example, 4 kHz thresholds have been found to be worse for MC females during the early-follicular phase (10.8 dB) compared to the late-follicular and the luteal phases (5.2 dB and 7 dB) (3).

A significant negative correlation has been found between efferent strength and estrogen level during the follicular phase: efferent strength was significantly lower in the late-follicular stage when estrogen levels are high than in the early-follicular stage when estrogen levels are low. Efferent strength is also significantly lower in the late-luteal stage than the early-follicular stage (when there are lower levels of both estrogen and progesterone). No significant correlation was found between efferent suppression magnitude and progesterone levels (49). There is just this one study looking at variations in efferent suppression magnitude across the

menstrual cycle, although the importance of estrogen in the maintenance of efferent suppression strength is shown by the decline in efferent suppression strength over 9 weeks in female CBA mice following estrogen suppression with tamoxifen which did not occur over that time frame in control animals (185). Like with the other aspects of auditory function there is inconsistent evidence showing fluctuation in OAE amplitude across the menstrual cycle. Some data suggests that TEOAE amplitude is largest during the early-follicular phase and smallest during the luteal phase (50) as cited in (133). Other data suggests that TEOAEs are slightly larger in the luteal phase than they are in the early-follicular phase (49). This group also found a small positive correlation between estrogen level and TEOAE amplitude during the follicular phase (49). There was no correlation between progesterone and TEOAE amplitude (49).

In contrast, other investigators have found no fluctuation in OAE amplitude across the menstrual cycle, with both DPOAE and TEOAEs found to be stable in different investigations (186, 187). Interestingly, no cyclic variation has been found in wave I latency which was stable across the menstrual cycle. (188). Wave I is generated by the distal auditory nerve and reflects the synchronous discharge of the auditory nerve fibres as they leave the cochlea and is consistent with the output of the cochlea (189). One research group suggests that the sex differences in OAE amplitude are due to organisational effects of hormones: the greater in-utero exposure of males to androgens permanently weakens their cochlear amplifiers and weakens their OAEs (55). This effect is suggested by research showing that females with male twins (who had greater pre-natal exposure to androgens) have smaller click-evoked OAEs than females with female twins (174). Also, female spotted hyenas which are exposed to high levels of androgens in utero and which are masculinised in many features do not have click-evoked OAEs that are significantly different from male spotted hyenas. Those spotted hyenas treated with anti-androgen agents prenatally have stronger OAEs (190). Other research has found that female pre-term infants have stronger TEOAEs from 35 weeks conceptional age (43), suggesting sex differences in OAE amplitude may be in place before the effects of circulating sex hormones come into play.

Finally, there are changes in cochlear ERα expression over the course of the mouse menstrual cycle. There was decreased ERα expression during the proestrous phase (where there are high levels of estrogen) when compared to the estrous and metestrous phase. There were no changes in ERβ expression over the cycle. Additionally, 17β-estradiol treatment decreased ERα expression but not ERβ (191). Based on these results showing changes in the expression of ERα during the mouse menstrual cycle and on other results showing the importance of ERβ in the protective response to NIHL (7), the authors hypothesise different roles for ERα and ERβ in the auditory system. They suggest that the role of ERα is to adjust auditory sensitivity based on the level of circulating hormones. ERβ is hypothesised to have a protective role, and comes into action when the auditory system is under stress such as responding to high levels of noise (191).

Do sex hormones act to effect susceptibility to NIHL via their effect on measures of auditory physiology or do they or act independently of these effects to effect susceptibility to NIHL?

While the weight of evidence does suggest an activational effect of sex hormones on auditory function, with the effects of estrogen more prominent, the effect of this relationship on sex differences in susceptibility to NIHL is unclear and has been little researched. The relationship in which females have their worse thresholds during the early-follicular phase and also have less TTS during this phase has led the authors speculate that perhaps female sex hormones act to alter thresholds and then it is the threshold itself which impacts on the amount of TTS (3). Suggesting this is the negative correlation between thresholds and TTS that has often been demonstrated, including male or mixed groups of participants (4, 27). However, females during the early-follicular phase had similar thresholds to OC females and males, but much less TTS whereas during the late-follicular and luteal phases female thresholds are better than OC and male thresholds but TTS is similar. This suggests that the female sex hormones also act independently of the threshold effect on TTS (3). Also suggesting this is the hypothesised different roles for ERα and ERβ in the auditory system, with the role of ERα to adjust auditory sensitivity based on the level of circulating hormones, with ERβ coming into play

and having a protective role when the auditory system is under stress such during high levels of noise (7, 191).

Summary and conclusion

The first part of this section examined the evidence for the presence of sex differences in the measures of auditory physiology that have been found to be successful predictors of susceptibility to NIHL. There is considerable evidence suggesting sex differences in PTA thresholds and OAE amplitudes although evidence suggesting sex differences in efferent suppression is more contradictory. There is no information available about how these sex differences in auditory physiology measures might impact on the prediction of susceptibility to NIHL. Given this, the third aim of the thesis is to determine whether the associations between auditory function measures (TEOAE amplitude, efferent suppression and 4 kHz threshold) and TTS and TEOAE shift differed in males and females.

There is also considerable evidence suggesting an activational effect of sex hormones on auditory function, although as with the association between sex hormones and NIHL there has been a reliance on the use of menstrual cycle phase to determine hormonal status. Given this, the fourth aim of the thesis is to determine whether there was an association between estrogen and progesterone levels and auditory function measures in female participants. Of particular interest is the association between the circulating levels of sex hormones and TEOAE amplitudes as there is significant conflict in the literature about whether apparent sex differences in OAE amplitude are related to activational or organisational effects of hormones.

Furthermore, given that there is little data from which to draw a conclusion about whether sex hormones effect susceptibility to NIHL through their effect on the auditory function measures or whether they act independently to influence NIHL, the fifth aim of the thesis is to determine whether estrogen and progesterone effected susceptibility to TTS and TEOAE shift through their effects on the auditory

function measures or whether they acted independently to effect TTS and TEOAE shift.

Summary and conclusion of literature review

In summary this literature review has found that while there is some evidence suggesting a sex difference in susceptibility to NIHL and in recovery from NIHL that there are inconsistencies. Perhaps the most important reason for these inconsistencies may be related to a lack of understanding of the impact of the hormonal status of the female participants on the results. There is some evidence showing an effect of sex hormones on NIHL but the human research has only used menstrual cycle stage as a marker of hormonal status rather than a measurement of hormonal levels which is problematic as there are large variations in hormone levels within the menstrual cycle stages. Most research into the relationship of sex hormones with NIHL has focused on estrogen so there is a severe lack of knowledge about the role of progesterone in NIHL.

Those aspects of auditory physiology that have been shown to be predictive of susceptibility to NIHL such as TEOAE amplitude, efferent suppression and 4 kHz threshold have been shown to differ in males and females and there is some evidence suggesting that there is an association between sex hormones and these aspects of auditory physiology. However, with one exception (49) there has been a focus on the use of menstrual cycle stage as a marker of hormonal status rather than direct measurement of hormonal levels. It is unclear from the literature whether estrogen and progesterone effect susceptibility to NIHL through their effects on baseline auditory physiology or whether they act independently to effect NIHL.

Resolution of these issues would further understanding of the nature of differences in susceptibility to NIHL and would also have implications for the management of NIHL through improved knowledge about the measurement of NIHL susceptibility. For example, if information about an individuals susceptibility to NIHL is to be effective in reducing NIHL it needs to be used to reduce individual noise exposures. A model for how information about individual susceptibility could be used to prevent workplace NIHL has been proposed by researchers at the South African Council for Scientific and Industrial Research Laboratory for Mining Innovation. They propose a two pronged approach for the prevention of NIHL which has a

focus on preventing hearing loss at both the macro and micro level (51). At the macro level they advise the development of a large database of audiometric results, dosimetry, and noise level measurements for each industry type. This can be used for analysis of trends in hearing levels amongst different occupational groupings within that industry. They can then be targeted for intervention such as hearing protection training and increased maintenance or engineering control of noise sources (51).

The micro-management of NIHL involves a focus on the quantifying the individual susceptibility to NIHL of workers by way of a "hearing loss risk matrix" – this can be used to target additional counselling, education and training at particularly susceptible individuals. This focus on individual risk acknowledges differences in susceptibility are substantial (25) and that a focus just on risk trends within the whole workforce could severely underestimate risk for some individuals. The matrix as conceived by these authors consists of four dimensions each with subdivisions that are scored individually (Figure 11). These include noise exposure factors, susceptibility factors (called medical in Figure 11), hearing protection factors and audiological factors.

			2	1	0	0	1	2			
1	Noise	Frequency	Low	Med	High	High	Med	Low	NCE	HCP	1
2		Intensity	High	Med	Low	High	Med	Low	HPD		2
3		Type	Impulse		Continuous	High	Med	Low	EMT		3
1	Medical	Toxins	Present		Absent	None	Some	Large	PLH shift	Audio	1
2		Solvents	Present		Absent	None	Some	Large	Notch shift		2
3		Smoking	Present		Absent	None	Some	Large	OAE shift		3
4		Alcohol	Present		Absent	None	Some	Large	Handicap		4
5		TB	Present		Absent						
6		HIV	Present		Absent						
7		OM	Present		Absent						
8		Heat	Present		Absent						
9		Exercise	Present		Absent						
10		Silicosis	Present		Absent						

Figure 11: Example of a hearing loss risk matrix. From (51).

Those who are at high risk for each factor in this matrix would score 2, those for whom the factor is absent would score 0. Those individuals who score above a particular value that is set in the workplace or industry could receive counselling

and explanation of those factors that place them at particular risk. They could receive more frequent audiological monitoring, re-evaluation of hearing protection type and training and be considered for other quieter jobs within the company (51).

The effective use of a matrix of this type requires significant research as to what susceptibility factors should be included, what weighting should be given to each factor and how the factors interact with each other (51). Information about the effect of sex and hormones on susceptibility could be used to help define these features. For example, if the presence of estrogen and/or progesterone is associated with a lesser risk of harm for females in all menstrual phases this would suggest that sex could be a factor in the "hearing loss risk matrix", with pre-menopausal females assigned a 1 or 0 to indicate their lesser risk.

Aims

Part 1

1. To determine whether males and females differed in TTS and TEOAE shift and recovery from TTS and TEOAE shift after exposure to a 3kHz pure-tone, 100 dB $L_{Aeq,}$ 15 minute, noise exposure.

2. To determine whether there was an association between estrogen and progesterone levels and TTS and TEOAE shift in female participants and whether males, OC females and females in the early-follicular, late-follicular and luteal phases differed in TTS and TEOAE shift.

Part 2

3. To determine whether the associations between auditory function measures (TEOAE amplitude, efferent suppression and 4 kHz threshold) and TTS and TEOAE shift differed in males and females.

4. To determine whether there is an association between estrogen and progesterone levels and auditory function measures in female participants.

5. To determine whether estrogen and progesterone effected susceptibility to TTS and TEOAE shift through their effects on the auditory function measures or whether they acted independently to effect TTS and TEOAE shift.

Methods

Overview

The methods section will begin with a summary of the facilities used in the research as well as details about participant recruitment and participants. It will then detail the procedures and tests used in part 1 of the research. This includes details of the noise exposure and how the individualised noise exposure levels were established, measurement of TTS and TEOAE shift and the blood testing procedures. The procedures and tests used in part 2 of the research will then be outlined. The section will end with information about data analysis.

Facilities

All audiometric testing and noise exposures were conducted in a sound-proof room with ambient noise levels below the maximum allowed for audiometric test rooms (192). The testing took place in the audiology department of the Greenlane Clinical Centre, a facility of Auckland District Health Board (ADHB). Audiological equipment had current calibration certificates. The TEOAE probe was checked regularly in a cavity using the ILO probe-check procedure to ensure proper stimulus levels. Testing took place over three sessions. Session one and two lasted for approximately two hours each and session three was around 45 minutes long.

Participant recruitment

Participants were recruited primarily through advertising on university campuses, word of mouth and through the participant recruitment website getparticipants.com (now called researchstudies.co.nz). Participants were also sought from advertisements in supermarkets, churches, shopping malls and sports clubs. Participants were offered Motor Trade Association (petrol) vouchers for their participation.

Participants

Participants were required to answer a set of pre-selection questions and were excluded if they had experienced significant occupational noise exposure for over 20 hours a week for over 3 months during their lives. Participants were also excluded if they answered often to having social noise exposures such as shooting,

playing in bands and musical instruments, using amplified music devices, attending loud music venues and using power tools and lawn mowers. Participants were also excluded if they had a family history of hearing loss, significant tinnitus or use of ototoxic medications. The complete question set is provided in appendix 1.

Participants had to have normal PTA thresholds (≤ 20 dB HL) at 500 Hz, 1 , 1.5, 2, 3, 4 and 6 kHz on the right side and a normal white noise threshold (125 Hz – 12000 Hz) on the left side. Thresholds were measured using insert earphones (EAR –tone 3A) and a calibrated audiometer (GSI 61 Clinical Audiometer) using the modified Hughson-Westlake technique (193). These measurements were obtained at the beginning of each session.

Participants also had to have a bilaterally normal otoscopic examination at each session with no obstructive wax. Participants also received tympanometry to exclude middle ear disorders. Tympanometry was performed with a GSI Tympstar using a 226 Hz probe tone. There were broad tympanometry inclusion criteria to exclude middle ear problems. Middle ear pressure (MEP) values had to be between -100 and +100 daPa (194), static admittance between 0.2 and 1.60 mmho (127), and estimated ear canal volume (ECV) between .6 and 1.8 mmho (127). One male participant (M18) had an ECV of 1.9 mmho on one occasion but as he had a clearly identifiable tympanometric peak was retained in the study. Another participant (M3) had a type C tympanogram on one occasion and testing was postponed to another day because of this.

The participant age range was initially set at 18-30 as some research had shown small changes in male hearing from 30 years of age (37). A change to an age range of 18-35 was made to increase the pool of potential participants given that hearing was still shown to be within the normal range for males and females at this age (37). In total, 28 female and 23 male participants between 18-35 were recruited for this study. One female participant was excluded from participation due to the presence of a previously unknown mild hearing loss. One female participant began the first session but then decided she no longer wanted to participate in the noise

exposure part of the protocol so testing was abandoned. One male participant completed the first session but did not return for the further sessions and another male participant left during the first session and did not return. Participant F25 is not included in any noise exposure analyses as an interruption to power supply occurred during the noise exposure and she was not able to return for a follow up session.

The full data set consists of 21 male and 26 female participants. There was no significant difference in the age of male and female participants, $t(45) = -.945$, $p = .350$, with a mean male age of 25.62 ± 4.83 SD and a mean female age of 26.80 ± 3.72 SD.

Part 1

Noise Exposure

Each participant was exposed to a 3 kHz continuous, pure-tone noise exposure of 100 dB L_{Aeq} for 15 minutes. The was generated by a GSI 61 Clinical Audiometer presented to the right ear through an insert ear-phone (EAR–tone 3A). This exposure had a frequency tolerance of ± 1%, the SPL tolerance was ± 3 dB and the rise and fall times did not exceed 200 ms (195). This exposure provided the equivalent energy to an eight-hour continuous A-weighted sound pressure level, $L_{Aeq,8h}$ of 85 dB which is the occupational safe noise exposure limit in New Zealand for an 8 hour work day as stated in Regulation 11 of the Health and Safety in Employment Regulations (1995) (118). One ear per person (the right) was investigated on the basis that this ear has been shown to have better hearing thresholds and stronger OAEs (65).

Studies of basilar membrane vibration show that the point of maximum vibration occurs half an octave above the frequency of stimulation at high intensity levels (162), so this noise exposure ensured a maximum TTS at around 4 kHz. This allowed comparison with previous TTS research which caused a 4 kHz TTS (1).

The transformation of dBHL values to reSPL values using the CDD+RECD transform

Individual variations in the characteristics of the ear canal and ear drum can lead to large individual variations in the SPL measured at the ear drum for a particular audiometer dBHL output (129, 130). Individual values of measured SPL near the ear drum for a given output have been shown to vary by as much as 20 dB in adults at 3 kHz using a ER-3A earphone (130).

To ensure that each participant received a 100 dB L_{Aeq} exposure at the ear drum an individualised dBHL to real-ear SPL (reSPL) transform was used. The 100 dB L_{Aeq} noise was converted into dB SPL by subtracting the A-weighting factor for 3 kHz (1.2 dB (196)). The reSPL value was calculated by adding the coupler to dial difference (CDD) and the real ear to coupler difference (RECD) to the noise level in dB SPL as per Munro and Davis (2003) (197) (see Figure 12).

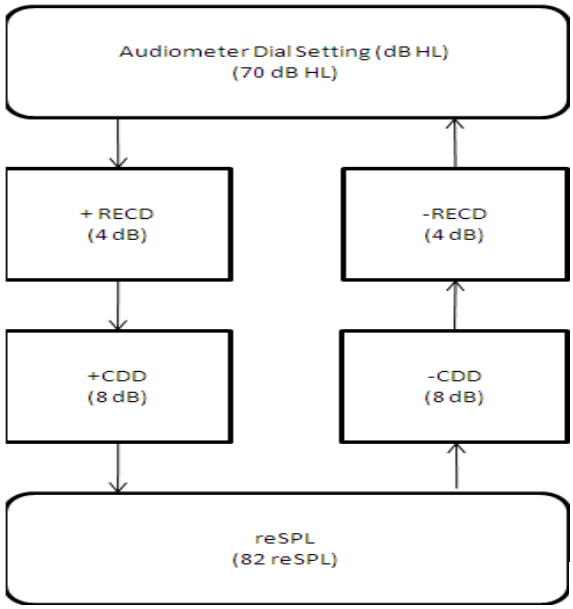


Figure 12: Illustration of the dB HL to reSPL transform showing the RECD and CDD acoustic transforms. The values are illustrative only. Adapted from (198).

Coupler to dial difference

The CDD was measured and re-measured after the audiometer received its annual calibration. The CDD is specific to the audiometer and so did not need to be measured separately for each participant. The CDD data is listed in appendix 2.

To measure the CDD the audiometer was set to an output of 70 dB HL and the output was measured in a Verifit® HA2 coupler using 500 Hz, 1 kHz, 2 kHz, 3 kHz and 4 kHz pure tones. The nipple of the ER-3A insert earphone was inserted into the tube of the HA2 coupler and the Verifit was put into sound level meter mode with a 1/12th octave filter. The difference between the dial reading and the reading in the coupler microphone box was the CDD. The test-retest reliability of the CDD was established by removing the insert nipple from the tube of the coupler and then replacing it and repeating the measurements at each frequency.

Real ear to coupler difference

The RECD was measured using the RECD function of the Verifit which generates a swept pure-tone signal. The initial step was measuring the coupler response, which required placement of the RECD transducer into an HA2 coupler. A coupler response curve was generated which showed the SPL in the coupler at each frequency.

The next step was measuring the real ear response. The probe tube was positioned in the right ear a fixed distance from the inter-tragal notch: 31 mm for males and 28 mm for females (197, 199). This placed the probe tube's tip within approximately 5-6 mm of the tympanic membrane, as the average adult ear canal length is around 24 mm in females and 25 mm in males (150) and the normal distance from the opening of the ear canal to the tragus is 10 mm (197). This gives accurate results up to 6 kHz of ± 2 dB (129) and this level of accuracy is maintained up to 4 kHz even if the probe tube is up to 9 mm away from the TM (200). Therefore this positioning gives accurate results up to 4 kHz, which was the frequency range of interest in this study.

Following otoscopy to ensure proper placement of the probe tube, the insert ear-phone and the RECD transducer were placed in the ear and the real-ear response was measured. The Verifit calculates the real ear response curve and the RECD curve and values, with the RECD curve being the difference in SPL between the coupler and the real ear response. The RECD measurement was repeated following

removal and reinsertion of the transducer, insert ear-phone and probe tube to establish the reliability of the measure.

A comparison was made between measured and predicted 3 kHz reSPL in the first 16 participants to ensure that predicted reSPL provided a good estimate of measured reSPL in these test conditions. Although a paired t-test showed a significant difference between the values: $t(15) = -7.652$, $p < .001$, with predicted reSPL larger than measured reSPL, the mean difference was only 1.03 dB ± 0.54 SD.

Transformation of the 99 dB SPL noise exposure level into dBHL resulted in audiometer presentation values ranging from 81 to 89 dBHL. There were no statistically significant sex differences in 3 kHz RECD values, $t(45) = 0.803$, $p = 0.426$, CDD+RECD transform value, $t(45) = 0.814$, $p = 0.420$, or in dBHL presentation value, $t(45) = 0.840$, $p = 0.405$ which was 85.57 dBHL ± 2.13 SD in males and 85.08 dBHL ± 1.90 SD in females.

TTS and TEOAE shift protocol

To calculate TTS and TEOAE shift, participants had pre and post exposure measurements of their 4 kHz thresholds (PTA) and TEOAEs. This was done in two parts: baseline measures during session one and experimental (post noise-exposure) measures during session two (see Figure 13). The baseline session had the same structure as the experimental session except that participants sat with the insert earphone in place but no sound. It was necessary to measure baseline PTA and TEOAE levels to estimate the levels and natural variation of thresholds, amplitudes and noise levels. Variation was likely due to practice effects, fatigue and boredom which would be expected to fluctuate over the time course of the session. For TEOAEs, it may reflect variation due to probe re-insertion. TEOAEs have been demonstrated to have good intra-subject reliability and the variation in OAE levels attributable to reinsertion of the probe tip has been found to be minimal in the same individual (201). However, to maintain consistency in the timing of the measurements across participants in this study there was minimal

time available for probe reinsertion, so a measure of variation due to probe reinsertion was vital.

Measurements in the two sessions were performed according to the schedule outlined in Figure 13. There was a break of a few hours to 66 days between session one and two; the length of this gap was determined by participant availability. The mean inter-session gap in females was 13.58 ± 17.20 days and in males was 12.19 ± 13.01 days. There was no significant difference, t (45) = 0.305, p = 0.762.

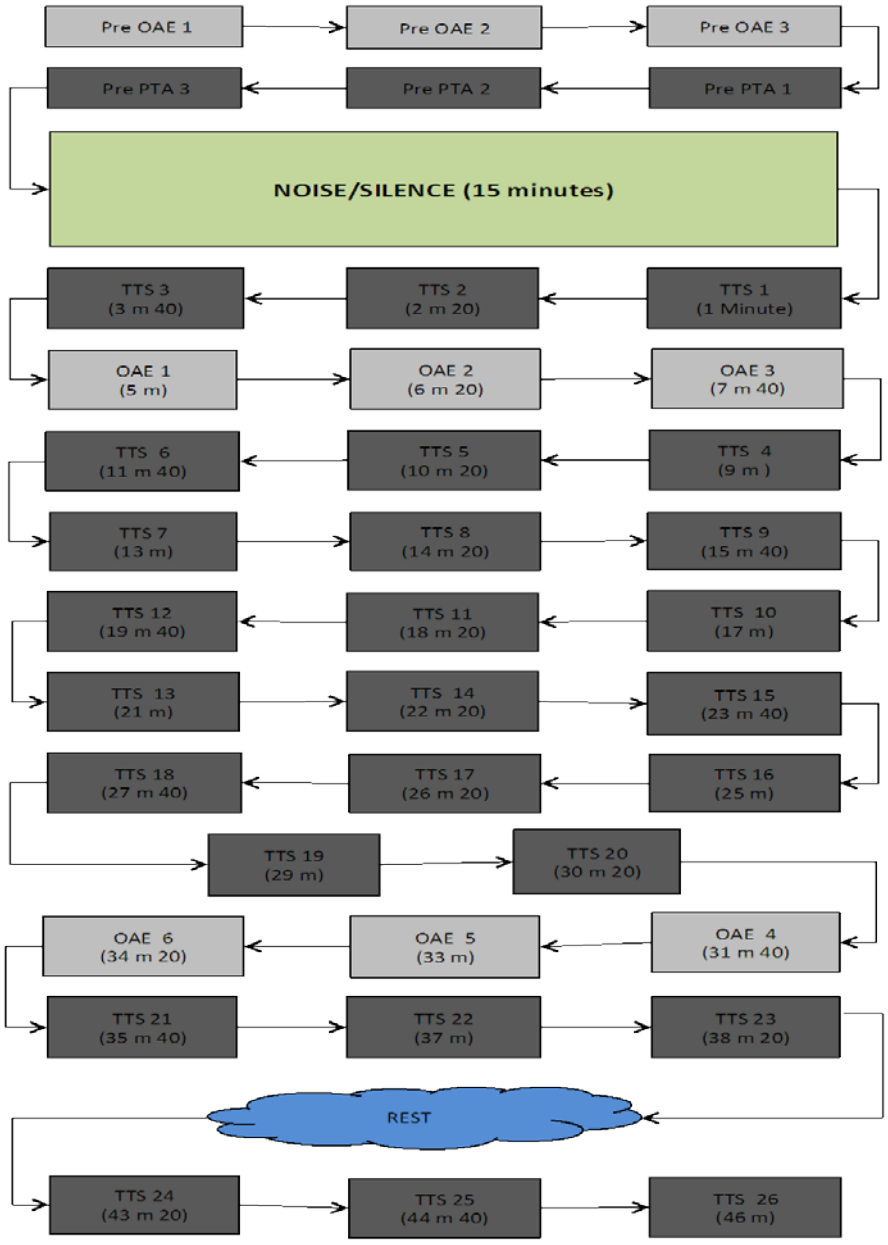


Figure 13: OAE and PTA schedule for session 1 (baseline session) and session 2 (noise exposure session).

The protocol began with three 1-minute OAE measurements, separated by 20-second gaps, followed by three 4 kHz PTA measurements. Following this, participants had a 15-minute noise exposure or no-sound period. Post exposure measurements began 1-minute post exposure with PTA and a new measure began every 1 minute and 20 seconds (80 seconds) after this. There were a total of 6 post exposure TEOAE measures and 26 PTA measures.

Initially the noise-exposure session protocol occurred according to a different protocol (Figure 14), however this was altered after 10 participants (M1-M5 and F1-F5) had been tested. The initial duration of post-exposure testing (34 minutes 20 seconds) was chosen because other studies with equivalent level noise exposures had indicated threshold and OAE recovery times of around this length (201, 202). However, recovery was incomplete by the final PTA measurement at 30 minutes 20 seconds, so it was decided to extend the testing time. Six additional TTS measurements were added to the end of the session, separated by a rest period. This brought the post-exposure testing time to 46 minutes.

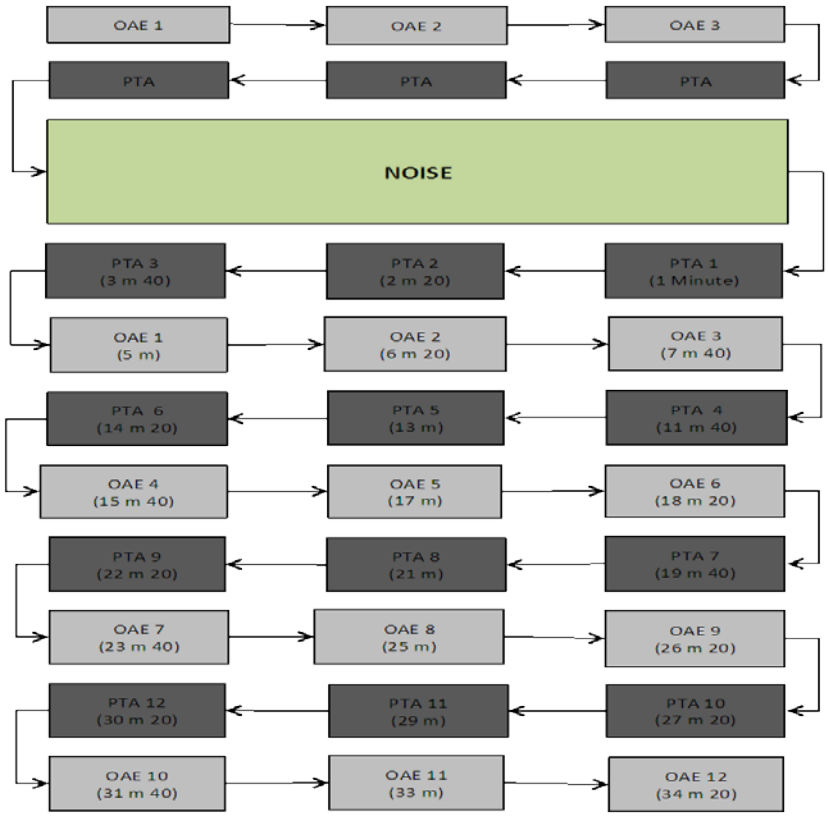


Figure 14: Initial OAE and PTA test schedule (noise exposure session)

Additionally, OAE measurements 4-9 were replaced with TTS measurements. This change was made because many participants had weak or un-measureable 4 kHz OAEs, making it difficult to measure a 4 kHz OAE shift. A post noise-exposure efferent suppression measure which was made between OAE 3 and PTA 4 was discarded due to difficulties in performing the measurement within the time allocated for it.

Furthermore, initially the baseline session had a different structure than the noise exposure session. The first three PTA and OAE measures occurred at the same time in the schedule, but the other measures were different due to the presence of the post-exposure efferent measure in the noise-exposure session and there were only three sets of PTA and OAE measures rather than four.

PTA measurement

4 kHz thresholds were obtained in 1 dB steps using a modified version of the Hughson-Westlake method. Threshold seeking began at 30 dBHL and the intensity was lowered by 10 dB until the participant ceased to respond. The intensity was then raised by 1 dB until another response was obtained and then intensity was decreased by two steps (2 dB) and increased by 1 step (1 dB) until the threshold was obtained in the normal manner. For further repetitions of threshold seeking the starting intensity was 4 dB above the last threshold.

TEOAEs

TEOAEs were selected as the OAE type for this research as they are the OAE type which has been most sensitive at detecting sex differences in OAEs (178). TEOAEs were obtained with an ILO 92 OAE system operating ILO88 v5.60y software. Recording began after sealing the probe in the ear canal and performing the probe check fit procedure to ensure as flat a frequency response as possible across the frequency range of 500 Hz to ~4.5 kHz. The click stimulus was adjusted to produce the target click level at the eardrum.

TEOAEs were recorded using the ILO default stimulus: an 80 μs click in the non-linear mode. The stimulus level was set to 80 dB SPL peak in the ear canal ± 2 dB (this equates to a stimulus level of around 40 – 45 dB SPL at each part of the cochlea) and TEOAEs were analysed in half octave bands (1, 1.4, 2, 2.8 and 4 kHz). The window began 2.5 ms after the stimulus presentation, which avoids acoustic ringing of the input stimuli and ends at 20 ms. The noise rejection level was set at 4.6 mPa/47.3 dB. TEOAE averaging was ceased manually after 1 minute (60 seconds) of recording.

Participants were seated upright in a chair and were advised about the importance of remaining quiet and as still as they could manage during OAE recording. Participants were able to read, use their phones and tablets and/or watch a silent DVD during the recording to reduce boredom-related fidgeting.

To maximise the data that could obtained, liberal data-inclusion criteria were used. TEOAEs were considered present if there was a SNR of 0 dB or better (26). Data also needed to have an amplitude of greater than -10 dB and appear above the noise floor on visual inspection for inclusion. When a pre-exposure TEOAE was measureable but a post-exposure measure was below the noise floor (SNR below 0 dB) then the post-exposure value was replaced with the noise level (as long as the noise value was below the pre-exposure TEOAE value; where it was higher, the measure was treated as missing)(26).

Calculating TTS and TEOAE shift

TTS was determined by calculating the difference in PTA thresholds measured before the noise exposure (pre-exposure) and those measured afterwards. This was calculated for each time point (Figure 13) to track recovery of the TTS over time. Temporary changes in TEOAEs were also calculated by measuring the differences in pre and post noise exposure values at each time point.

Blood test procedures

Female participants had blood tests to determine the levels of the sex hormones 17-β estradiol (estrogen) and progesterone. Hormone levels were not tested in men, because their estrogen and progesterone levels are very low and relatively stable (53, 203, 204). Blood tests were conducted on the day of testing, and time between blood collection and the beginning of the noise exposure session varied between approximately half an hour to no more than 12 hours.

The estrogen and progesterone assays were both a delayed one-step serum immunoassay using Chemiluminescent Microparticle Immunoassay (CMIA) technology using flexible assay protocols called Chemiflex (203, 204). The maximum amount of blood that was drawn from participants was 1 SST tube or 8.5 ml. The blood samples were processed by Diagnostic Medlab using a ARCHITECT *i* System by Abbott. The precision of the progesterone assay was ≤ 10% of the total coefficient of variation (CV) for low values and ≤ 7% of CV for medium and high values. The sensitivity was ≤ 0.318 nmol/l (204). The precision of the estrogen assay was ≤ 18.35 pmol/l for low values and ≤ 7% of CV for medium and high values. The sensitivity was ≤ 36.7 pmol/l (203).

Due to the inaccuracies inherent in using estimates of menstrual cycle stage to assess the effect of estrogen and progesterone on function (24) this study measured estrogen and progesterone directly. To enable comparison with previous research that has focused on menstrual cycle stage, menstrually-cycling participants were classified into either the early-follicular, late-follicular or luteal phase based on a comparison of their measured estrogen and progesterone values to the range of estrogen and progesterone values that have been measured during the different menstrual cycle phases (see Table 1 and Table 2).

Due to the large variability in hormone levels that can occur in the different menstrual cycle phases a conservative approach was taken when classifying participants into the different menstrual cycle groups. Those with progesterone levels that were too low to be within the range of expected values in the luteal phase (below the lowest 2.5 centile figure from the luteal phase (1.62 nmol/l – see

Table 2)), and estrogen levels that were below the 2.5 centile figure from the late-follicular phase (111.67 pmol/l – see Table 1) were classified into the early-follicular period.

Those with progesterone levels that were below the range of expected values in the luteal period and estrogen levels that were above the range of expected values in the early follicular period (above the 97.5 centile figure of 613.6) were classified into the late-follicular phase.

Those with progesterone levels that were above the range of expected values in the follicular phase (above the highest 97.5 centile figure from the follicular phase of 5.22 nmol/l) were classified into the luteal phase.

Estrogen	Mean	2.5 Centile	Median	97.5 Centile
Early F	211.4	42.9	178	613.6
Late F	720.4	116.7	615.5	2051.9
Early L	473.4	115.6	443.7	1079
Mid L	746.9	214	776.6	1256.2
Late L	487.4	59.1	435.6	1275

Table 1: Range of estrogen levels during the different phases of the menstrual cycle (pmol/l). F = follicular and L = luteal. Reproduced from (145).

Progesterone	Mean	2.5 Centile	Median	97.5 Centile
Early F	1.84	.016	1.56	5.22
Late F	1.59	.06	1.40	4.26
Early L	14.28	2.58	12.72	34.41
Mid L	35.27	21	35.20	53.74
Late L	17.11	1.62	14.21	46.46

Table 2: Range of progesterone levels during the different phases of the menstrual cycle (nmol/l). F = follicular and L = luteal. Reproduced from (145).

Those participants who could not be unambiguously allocated to either of these three groups were not included in these group analyses. This included 4/26 participants in session 2 and 5/21 available participants in session 3. Participants' estrogen, progesterone, OC, pregnancy status and menstrual phase allocation is described in Appendix 3.

Part 2

Part 2 measured sex differences in measures of auditory physiology, correlations of auditory physiology measures with the TTS and TEOAE shift from part 1 and correlations of estrogen and progesterone with auditory physiology measures. The methods for measuring TEOAE amplitude, 4 kHz threshold and sex hormone levels have been previously outlined in part 1.

Efferent suppression

Efferent suppression measures were obtained at the beginning of session two before beginning the noise exposure protocol to investigate the persistence of the efferent effect in males and females as well as efferent suppression magnitude.

The protocol is outlined below (Figure 15). Both the baseline and efferent stimulus conditions contained eight 1-minute TEOAE recording intervals, interspersed with 20-second non-recording intervals. In the efferent condition the white noise was introduced to the left ear from the time of onset of the 1st OAE recording until the onset of the 6th OAE recording (6 m 40s). The post efferent recording interval consisted of three 1-minute long TEOAE recording intervals. This length of testing was chosen to investigate the persistence of efferent stimulation for as long as possible while keeping the total length of the test session to around two hours.

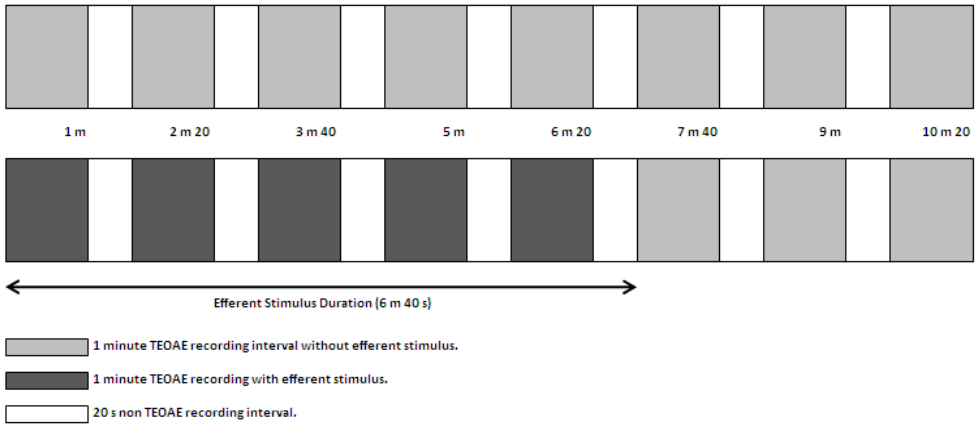


Figure 15: Session two efferent suppression protocol. TEOAEs are recorded in the right ear and the efferent stimulus is presented to the left ear.

The protocol was based on van Zyl (2009), although this protocol has shorter control and efferent conditions (10 minutes 20 seconds as opposed to 20 minutes),

with shorter gaps between TEOAE recordings. There was a 5-minute break from auditory stimulation between the two conditions, although the OAE probe was not removed during this time.

TEOAEs were recorded at 70 dB ± 2 dB in the right ear using the same system as listed in the previous section. The efferent system stimulus was a contralateral white noise (250 Hz -5000 Hz) (205), delivered to the left ear by a GSI 61 audiometer through an insert ear phone at 40 dB sensation level (SL) (range: 30 dBHL to 45 dBHL) . The sensation level was obtained by calculating the average of the left ear white noise threshold from the 1st and 2nd sessions and adding 40 dB. The stimulus level of 40 dB SL is below the level at which acoustic crossover and stimulation of the acoustic reflex has been shown to occur (206, 207). The mean male acoustic reflex threshold was 79.76 dB ± 8.14 (range 65 to 95 dBHL) and mean female threshold was 78.65 dB ± 7.94 (60 to 90 dBHL). Figure 16 illustrates the pathways involved in the stimulation of right efferent system by the contralateral efferent stimulus.

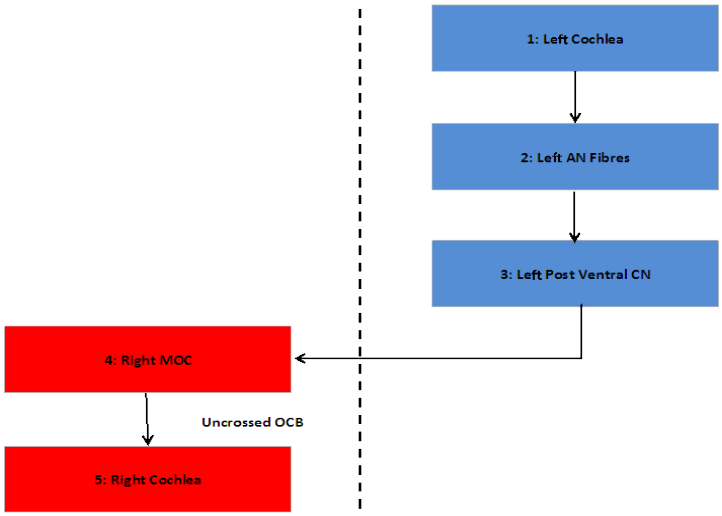


Figure 16: Schematic showing the pathway of suppression of right-sided OAEs by the suppressor noise presented to the left ear. AN = auditory nerve, CN = cochlear nucleus, of the stages involved in the contralateral efferent pathway as measured in the right ear. Adapted from (74).

Following the efferent suppression measurements participants were given a minimum ten minute break to relax and to allow the auditory system to recover from any lingering effects of efferent stimulation before beginning the noise exposure protocol.

Session three

Whilst the study was underway a third test session was added to the initial two test sessions. This allowed the association of TTS and OAE shift with a larger range of OAE values and efferent suppression values. Female participants had another blood test for estrogen and progesterone.

Those participants who had already been recruited were invited to attend the third session and offered an additional petrol voucher. Female participants were provided with a blood test form in the mail or given the form at their second session so that they could have their blood test prior to the session. In total 17/21 male participants and 21/26 female participants took part in the third session. There was a gap of between 3 and 488 days between session two and three. The mean inter-session gap in females was 179.48 ± 163.55 days and in males was 127.65 ± 159.80 days. There was no significant difference between the time gap for males and females ($t(36) = 0.981$, $p = 0.333$).

TEOAEs were measured at 60, 65, 70, 75 and 80 dB SPL ± 2 dB. The order of testing was randomised for each participant by placing cards representing the stimulus levels in a box and drawing them at random. At each stimulus level a one minute TEOAE recording was made to be consistent with the TEOAE measurements from the earlier session. This was followed by a one minute break. A second TEOAE recording was then made with the same contralateral stimulus as the previous sessions. The efferent stimulus was presented from the onset to the offset of the TEOAE recording. There was then a break of at least one minute before recording at the next stimulus level.

A single measurement was made at each intensity level to try and keep the session as short as possible (around 45 minutes) while testing as large a range of intensities as possible. It was felt that participant return rate, particularly for those initially recruited for two sessions would be greater with a shorter session. If participants caused too much noise, the measurement would be restarted.

Data analysis

Data was analysed using a combination of statistical tests, including as necessary Pearson's correlations, paired t-tests, independent samples t-tests, one-way ANOVA and repeated measures (RM) ANOVA with a between-subjects factor of sex. Analyses were performed using SPSS 18 and 20. Degrees of freedom of all analyses were corrected with a Greenhouse-Geisser (GG) correction when Mauchly's test of sphericity was significant. Pair-wise comparisons were made with a Bonferroni correction. With all one-way ANOVA analyses Welch's F was used when homogeneity of variance requirements were not met, with Games-Howell post-hoc tests. Bonferroni post-hoc tests were used otherwise.

All error bars on graphs are 1 standard error of the mean (SEM) and in the text values are listed as mean ± standard deviation (SD) unless otherwise mentioned.

General auditory function

- Right ear PTA was analysed with a RM ANOVA with RM factors of frequency and session. N = 21 female, 17 male.
- Immittance measures (admittance, MEP, volume) were analysed with a RM ANOVA with RM factors of ear and session. N = 21 female, 16 male.
- The contralateral acoustic reflex threshold was analysed with an independent samples t-test. N = 26 female and 21 male.

Differences between males and females in TTS, OAE shift and recovery

Comparisons were made between the pre-exposure PTA and OAE measurements from the baseline session and those from the noise exposure session (see Figure 13). This was to determine whether to use the mean of all pre-exposure values when calculating TTS and OAE shift or whether to use the mean of the three pre-exposure measurements from the noise-exposure session. If there was a significant difference between them then the mean of the three pre-exposure measurements from the noise-exposure session would used on the basis that these would reflect the most current physiological state. If they were not significantly different then the mean of all pre-exposure measures would be used on the basis that averaging

across more measures would provide a more accurate representation of the true value.

- These comparisons were made with a RM ANOVA with pre-exposure time as the RM factor. The N of the pre-exposure 4 kHz threshold was 17 female and 16 male. The N of the pre-exposure 4 kHz TEOAE analysis was 14 female and 9 male.
- Initial TTS which occurred at 1 minute post-exposure (TTS 1) and initial OAE-shift which occurred at 5 minutes post-exposure (OAE shift 1) were analysed with independent samples t-tests. The N of TTS 1 was 25 female and 21 male. The N of OAE shift 1 was 22 female and 17 male.
- TTS and OAE shift recovery were analysed with RM ANOVA with post-exposure time as the RM factor. The N of the TTS recovery group was 19 female and 16 male. The N of the OAE shift recovery group was 19 female and 14 male.

Association between estrogen, progesterone and TTS, OAE shift and recovery

Associations between estrogen, progesterone, TTS 1 and OAE shift 1 were assessed with Pearson's correlations. The log of progesterone data was used in these calculations due to the shape of the progesterone distribution. The non-parametric Spearman's rank correlation test was considered as an alternative, however as there were no meaningful differences in the outcomes of these tests the parametric Pearson's correlations were used to be consistent with the other statistical tests. The N's of these correlations are listed in Table 4.

Additionally, participants were classified into five groups: early-follicular, late-follicular, luteal, OC and male. Additional comparisons were made between these groups which are termed hormone group analyses.

- Analysis of TTS 1 and OAE shift 1 in these hormone groups was performed using one-way ANOVA. The N's for TTS 1 were early-follicular 5, late-follicular 2, luteal 5, OC 5 and male 21. The N's for OAE shift 1 were early-follicular 4, late-follicular 2, luteal 4, OC 5 and male 17.
- Analysis of TTS and OAE shift recovery in the hormone groups used RM ANOVA with post-exposure time as the RM factor. The N's for the TTS

recovery group were early-follicular 5, late-follicular 2, luteal 5, OC 5 and male 21. The N's for the OAE shift recovery group were early-follicular 4, late-follicular 2, luteal 2, OC 5 and male 14.

The association of auditory physiology measures with TTS and OAE shift in males and females

Analysis of efferent suppression magnitude included the overall amplitude OAE as it had the highest values and had the least number of missing values.

The associations between efferent suppression magnitude (60, 65, 70, 75 and 80 dB), 4 kHz OAE amplitude (60, 65, 70, 75 and 80 dB) and 4 kHz threshold with TTS 1 and OAE shift 1 were assessed with Pearson's correlations. The N's of these correlations are listed in Table 4.

The association of auditory physiology measures with estrogen and progesterone

The associations between estrogen, progesterone with efferent suppression magnitude (60, 65, 70, 75 and 80 dB), 4 kHz OAE amplitude (60, 65, 70, 75 and 80 dB) and 4 kHz threshold were assessed with Pearson's correlations. The N's of these correlations are listed in Table 5.

- Differences between the hormone groups in efferent suppression magnitude were assessed with RM ANOVA with OAE stimulus as RM factor. The N's were early follicular 2, luteal 5, OC 5 and male 11.
    - Differences between males and females in efferent suppression magnitude (60-80 dB) were assessed with RM ANOVA with OAE stimulus level as the RM factor. Female N was 18 and male was 11.
- Hormone group differences in OAE amplitude were assessed with RM ANOVA with OAE stimulus level as the RM factor. The early-follicular N was 5, late-follicular was 2, luteal 6, OC 5 and male 13.
    - Differences between males and females in OAE amplitude (65-80 dB) were assessed with RM ANOVA with OAE stimulus level as the RM factor. Female N was 20 and male was 10.

- Differences between the hormone groups in 4 kHz threshold were assessed with one-way ANOVA. The early-follicular N was 5, late-follicular was 2, luteal 5, OC 5 and male 21.

Linear regression models

To assess the role of efferent suppression magnitude (60, 65, 70, 75 and 80 dB), 4 kHz OAE amplitude (60, 65, 70, 75 and 80 dB) and 4 kHz threshold in mediating the relationship between estrogen and progesterone on TTS and OAE shift, linear regression models were produced. A separate model was produced to assess the mediating role of each auditory function measure on TTS and on OAE shift, so each model includes all those participants who had data available for that auditory function measure and either TTS or OAE shift data meaning that participant numbers differed slightly between models. Each model had three levels: the first level looked at the effect of estrogen alone on TTS and OAE shift. The second looked at the effect of estrogen and progesterone together on TTS and OAE shift and the third looked at the effect of estrogen and progesterone as well as the auditory measure on TTS and OAE shift (Table 3).

		TTS Models	OAE Shift Models
Hormones Alone		16	13
Efferent Suppression	60 dB	11	10
	65 dB	11	10
	70 dB	13	11
	75 dB	13	11
	80 dB	13	11
OAE Amplitude	60 dB	11	10
	65 dB	10	10
	70 dB	14	13
	75 dB	12	11
	80 dB	11	12
4 kHz Threshold		16	13

Table 3: Participant numbers for the linear regression models.

Ethical approval

Ethical approval for this study was obtained through the Northern X regional Ethics Committee. This was notified to the Massey University Human Ethics Committee. Institutional approval to perform research on ADHB premises was obtained from the ADHB research office.

Results

Baseline auditory function

Males and females showed no difference in PTA thresholds (500 Hz -6 kHz): F (1, 36) = 0.052, p = 0.821. There was no interaction between sex and frequency: F (5, 180) = 0.1111, p = 0.990 or between sex and session: F (2, 72) = 0.147, p = 0.863 (Figure 17).

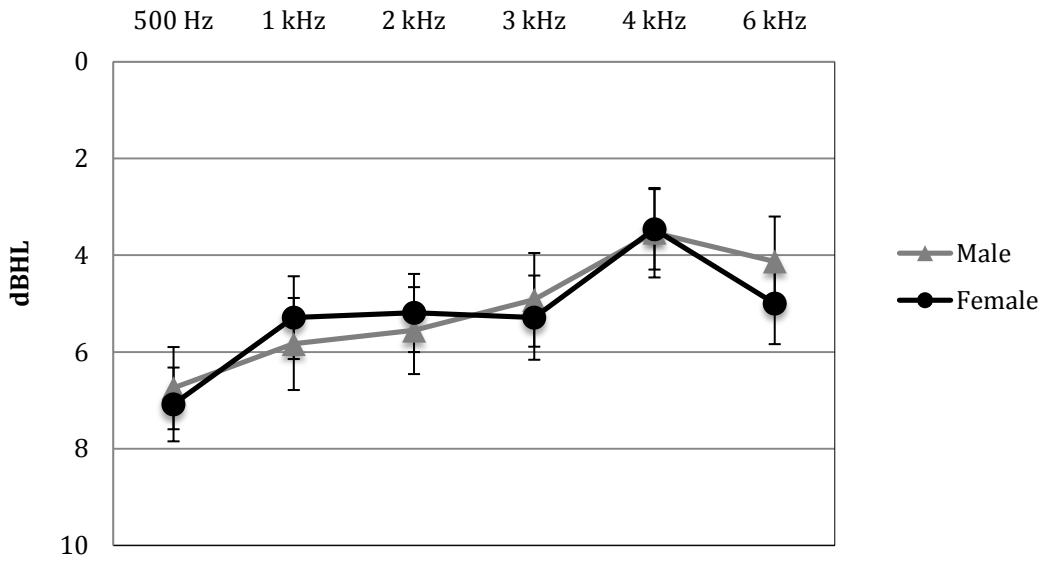


Figure 17: Male and female PTA thresholds (5 dB step-size).

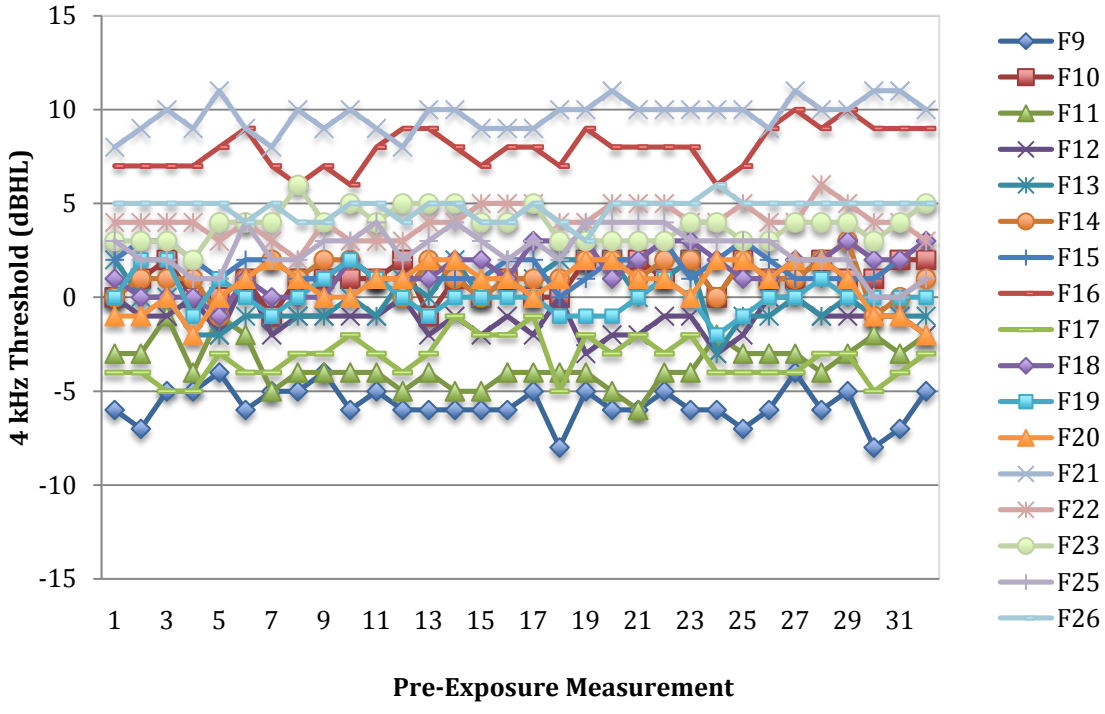
There were no differences between the sexes in admittance: F (1, 35) = 0.042, p = 0.839. There was also no interaction between ear and sex: F (1, 35) = 0.594, p = 0.446 or between session (1-3) and sex: F (2, 70) = 1.186, p = 0.312. Additionally, there was no main effect of sex on volume: F (1, 35) = 1.723, p = 0.198, no interaction between ear and sex on volume: F (1, 35) = 0.264, p = 0.611, or between session and sex: F (2, 70) = 1.401, p = 0.253. There was no main effect of sex on MEP: F (1, 33) = 1.608, p = 0.214, no interaction of sex and ear: F (1, 33) = 0.163, p = 0.689, or of sex and session on MEP: F (2, 66) = 0.344, p = 0.710. There was no difference between males and females in the contralateral BBN AR thresholds: t (45) = 0.470, p = 0.640.

Aim 1: To determine whether males and females differed in TTS and TEOAE shift and recovery from TTS and TEOAE shift after exposure to a 3kHz pure-tone, 100 dB L_{Aeq}, 15 minute, noise exposure

Pre-exposure 4 kHz thresholds

There were some isolated differences in threshold across the baseline session; F (31, 961) = 2.288, p = 0.019, however, there was no systematic change (individual data in Figure 18 and group averaged data in Figure 19). There was no main effect of sex on threshold during the baseline period, F (1, 31) = 0.000, p = 0.987 and no interaction of sex and time, F (31, 961) = 0.935, p = 0.492. The mean female pre-exposure threshold was 1.469 dBHL ± 3.915 and the mean male pre-exposure threshold was 1.443 dBHL ± 4.945.

As pre-exposure thresholds did not systematically increase or decrease over the course of the baseline period for either males or females (Figure 18 and Figure 19) it was decided to use the average of all pre-exposure thresholds when calculating TTS.



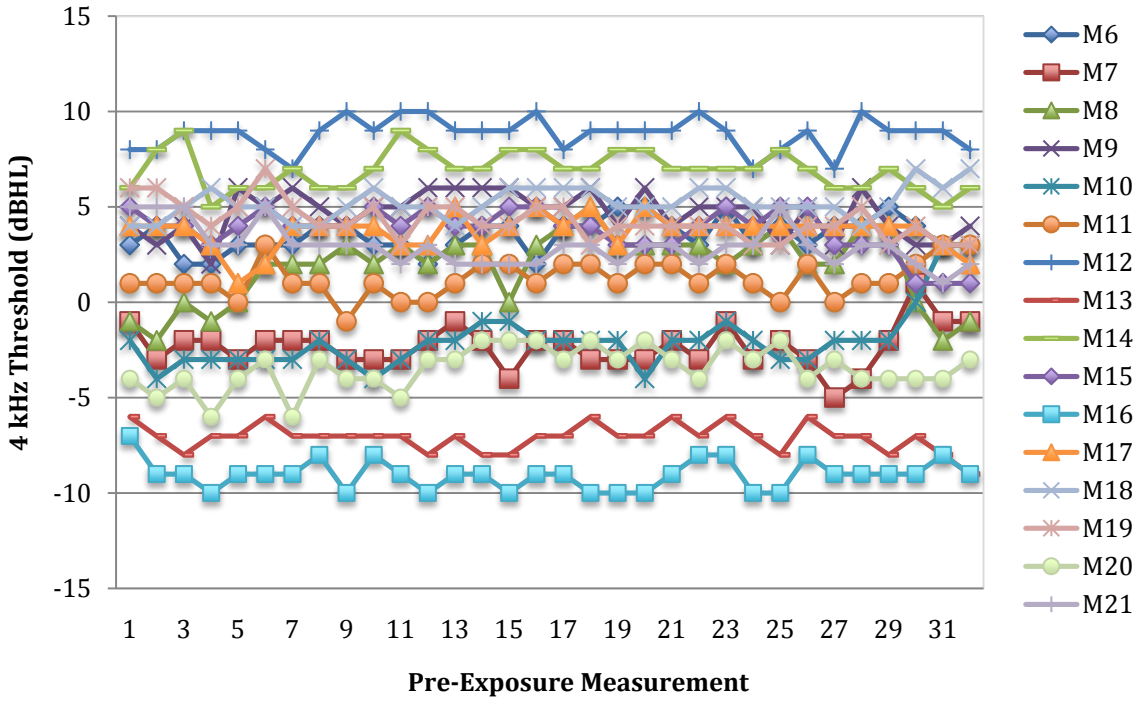


Figure 18: Individual 4 kHz pre-exposure thresholds at the 32 baseline measurement times (measurements 30, 31 and 32 occurred at the beginning of session 2) for males (bottom) and females (top).

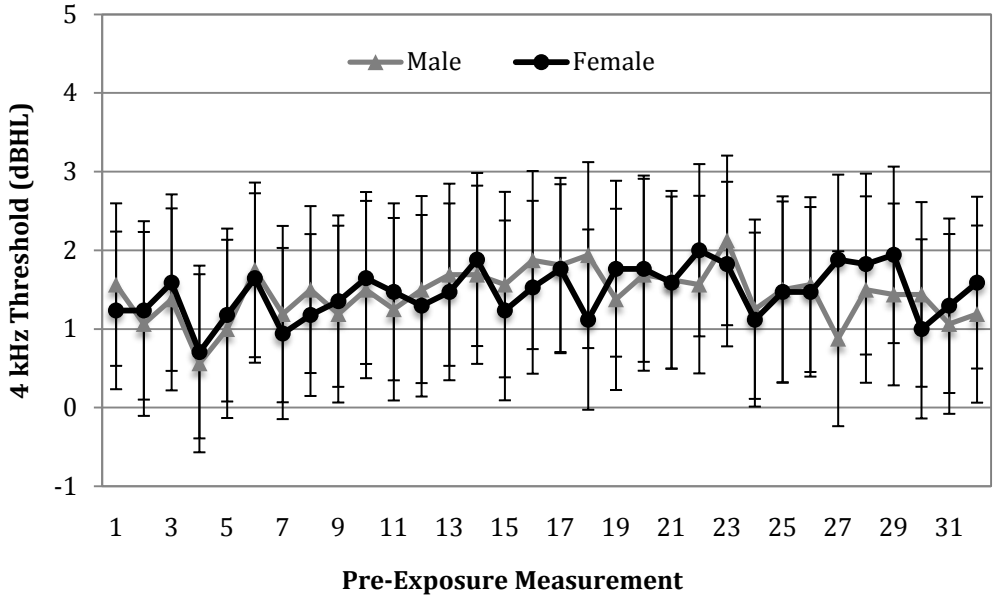


Figure 19: Male and female 4 kHz pre-exposure thresholds at the 32 baseline measurement times (measurements 30, 31 and 32 occurred at the beginning of session 2).

TTS 1

There was no significant difference in TTS at one minute post-exposure (TTS 1) between the sexes: t (44) = -0.447, p = 0.657 (see Figure 20). TTS 1 for females varied from 2.34 dB to 21.31 dB with a mean of 11.68 dB ± 6.01. TTS 1 for males varied from 2.5 dB to 23.44 dB with a mean of 12.47 dB ± 5.94.

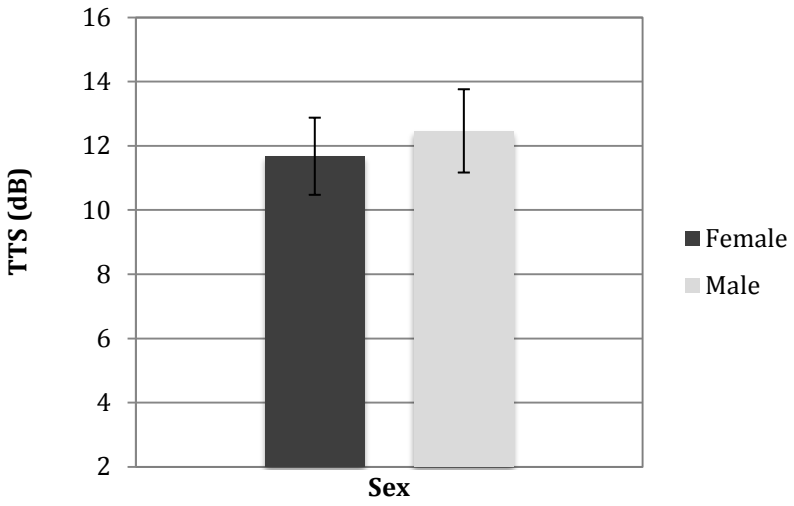
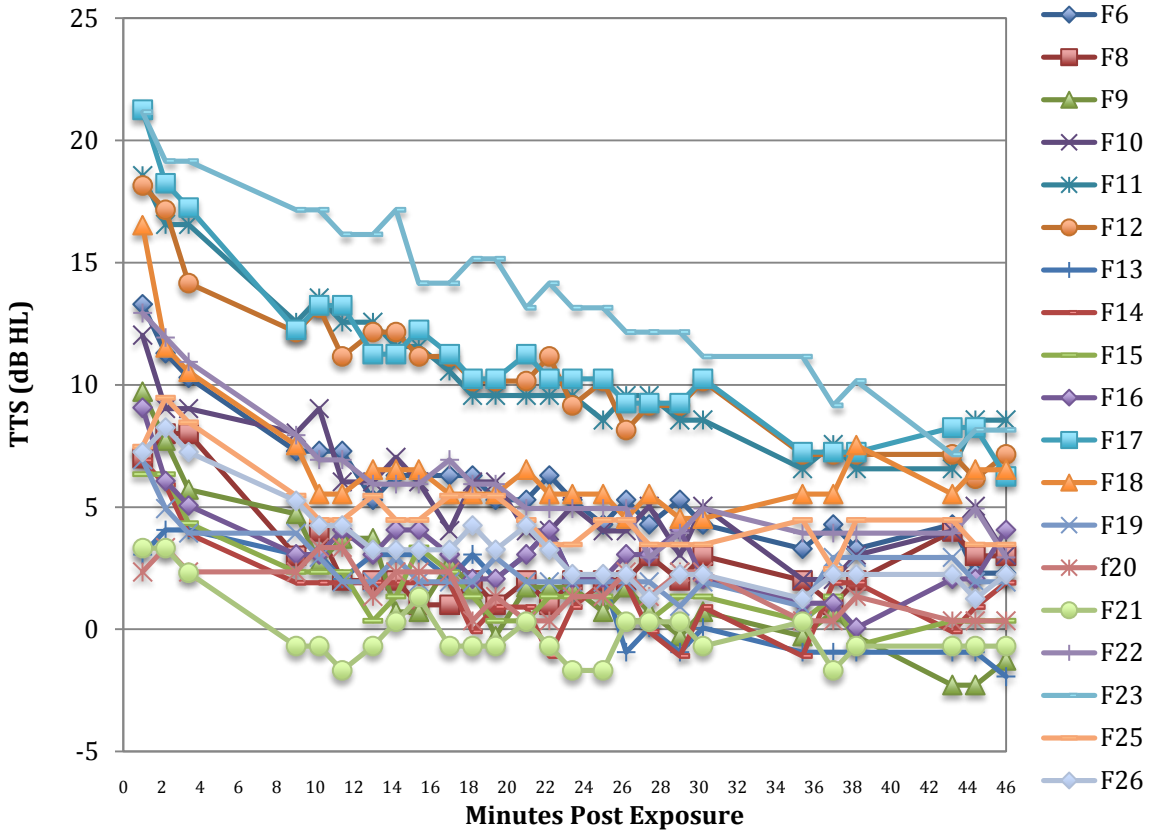


Figure 20: TTS 1 in males and females.

TTS recovery

TTS recovery was measured to 46 minutes post exposure. There was no main effect of sex: F (1, 33) = 0.562, p = 0.459 on TTS over the 46 minute period, however there was a significant interaction between sex and post-exposure time on TTS: F (25, 825) = 3.196, p = 0.030. Examination of the recovery curves (individual data in Figure 21 and group averaged data in Figure 22) shows that female TTS is somewhat less than male TTS at the beginning (male = 12.056 dB ± 6.201 and female = 10.701 dB ± 6.035), whereas it is greater than male TTS from 9 minutes onwards. Male TTS at 46 minutes was 1.56 ± 2.668 dB and final female TTS was 3.07 ± 3.100 dB. This indicates a slower recovery from TTS in females than in males.



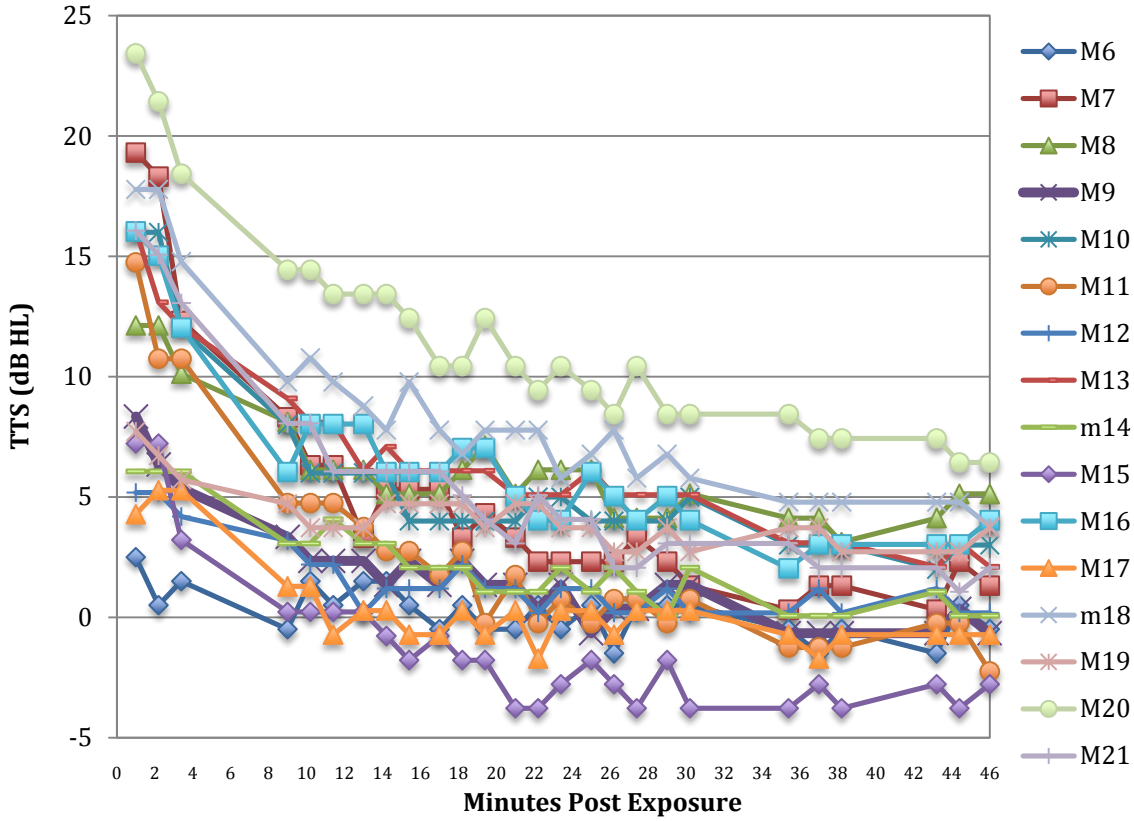


Figure 21: Individual TTS recovery curves between 1 minute and 46 minutes post-exposure for males and (bottom) and females (top). Scale is in seconds.

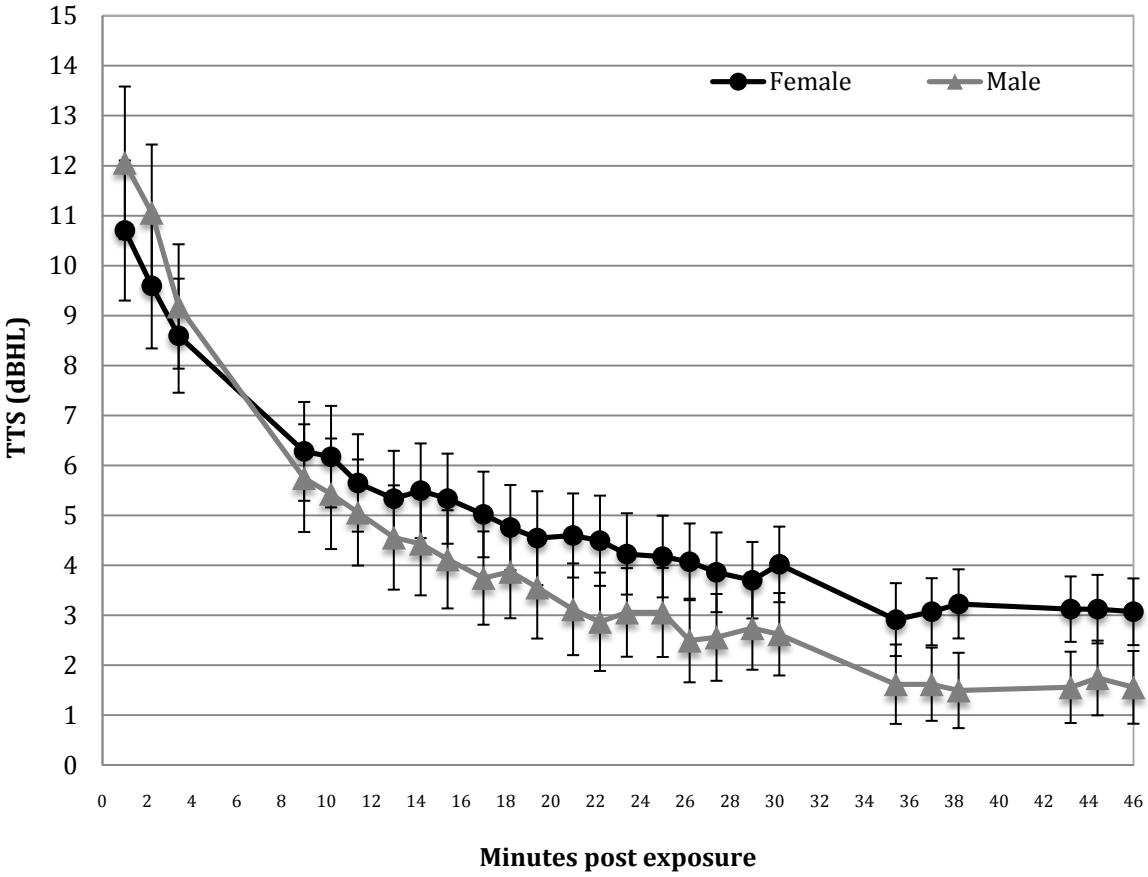


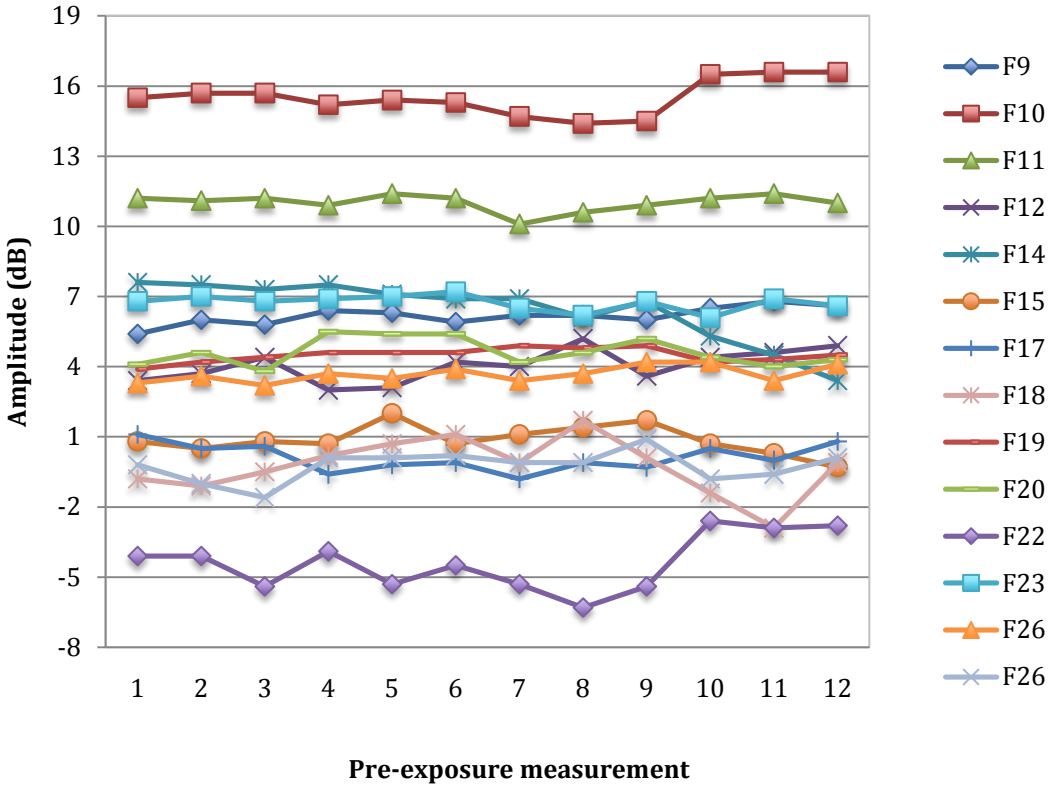
Figure 22: TTS recovery between 1 minute and 46 minutes post-exposure for males and females.

Examination of the TTS 1 data and TTS recovery data shows that there was no sex difference in TTS 1 but there was a difference in recovery from TTS.

Pre-exposure 4 kHz TEOAE amplitude

There was no variation of OAE amplitude across pre-exposure time: $F(11, 231) = 1.506$, $p = 0.217$ and no difference between the sexes in pre-exposure OAE amplitude ($F(1, 21) = 0.836$, $p = 0.371$). There was also no pre-exposure time by sex interaction: $F(11, 231) = 0.619$, $p = 0.622$.

As pre-exposure amplitudes did not systematically increase or decrease over the course of the baseline period for either males or females (individual data in Figure 23 and group averaged data in Figure 24) it was decided to use the average of all pre-exposure amplitudes when calculating OAE shift.



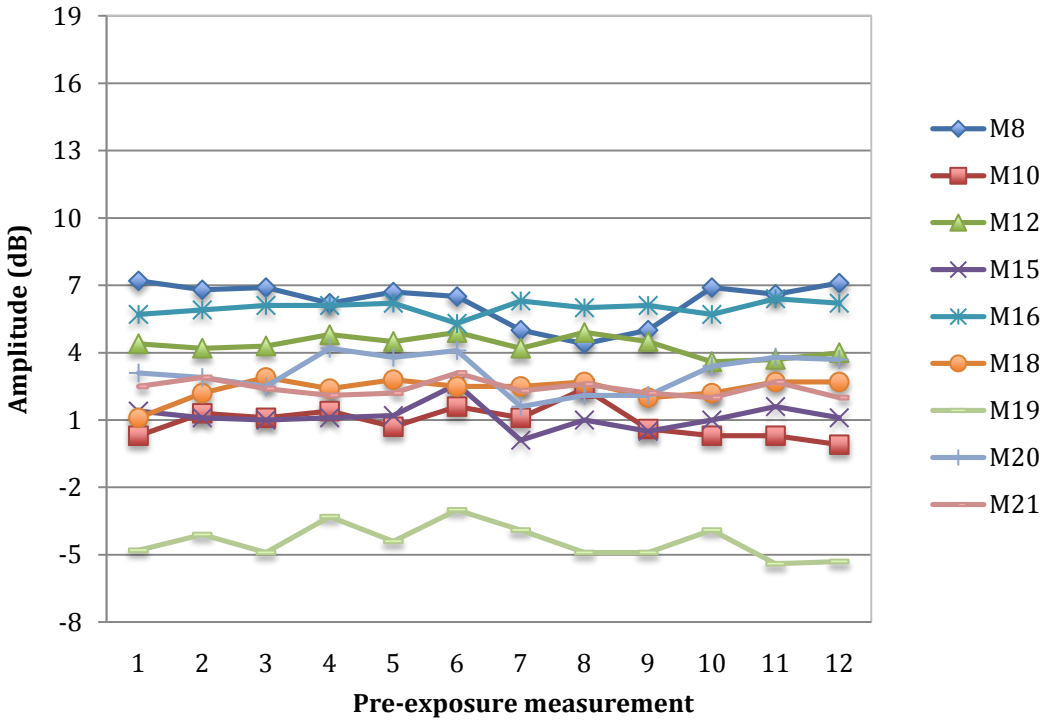


Figure 23: Individual 4 kHz pre-exposure TEOAE amplitudes for males and females.

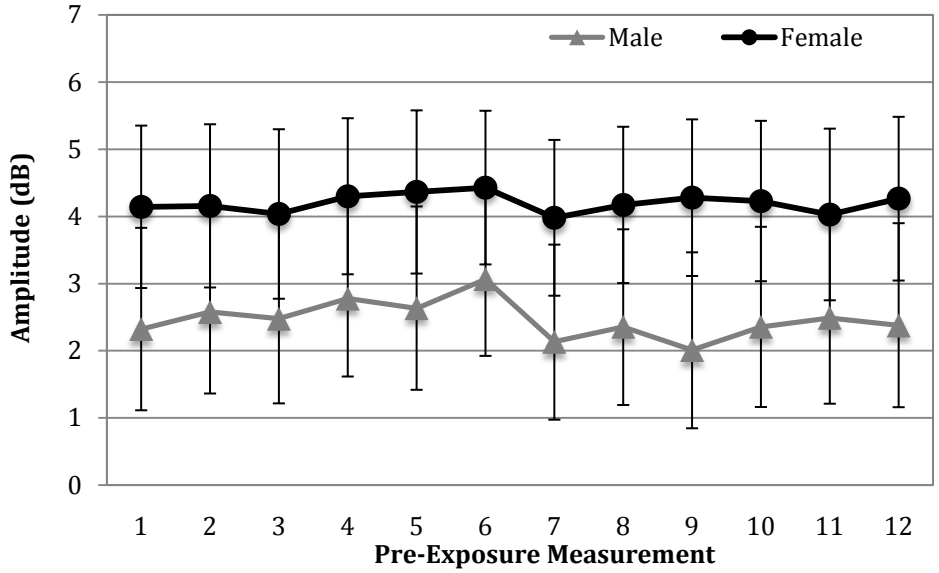


Figure 24: Pre-exposure 4 kHz OAE amplitude for the 12 pre-exposure measurements. Measurements 10, 11 and 12 are from session two.

OAE shift 1

Analysis of OAE shift 1 (5 minutes post-exposure) showed no significant difference between males and females: t (37) = 1.163, p = 0.520 (Figure 25). OAE shift 1 in females was 2.23 dB ± 1.69 and in males was 1.66 dB ± 1.23.

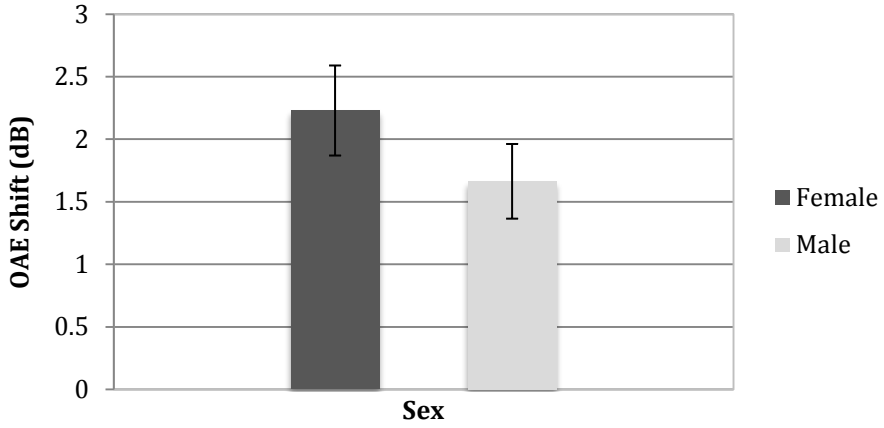


Figure 25: OAE shift 1 in males and females.

OAE shift recovery

There was no main effect of sex on OAE shift over the post-exposure time: F (1, 31) = 0.860, p = 0.361. There was also no interaction between sex and post-exposure time on OAE shift: F (5, 155) = 0.380, p = 0.780 (see Figure 26 for individual data and Figure 27 for averaged recovery data).

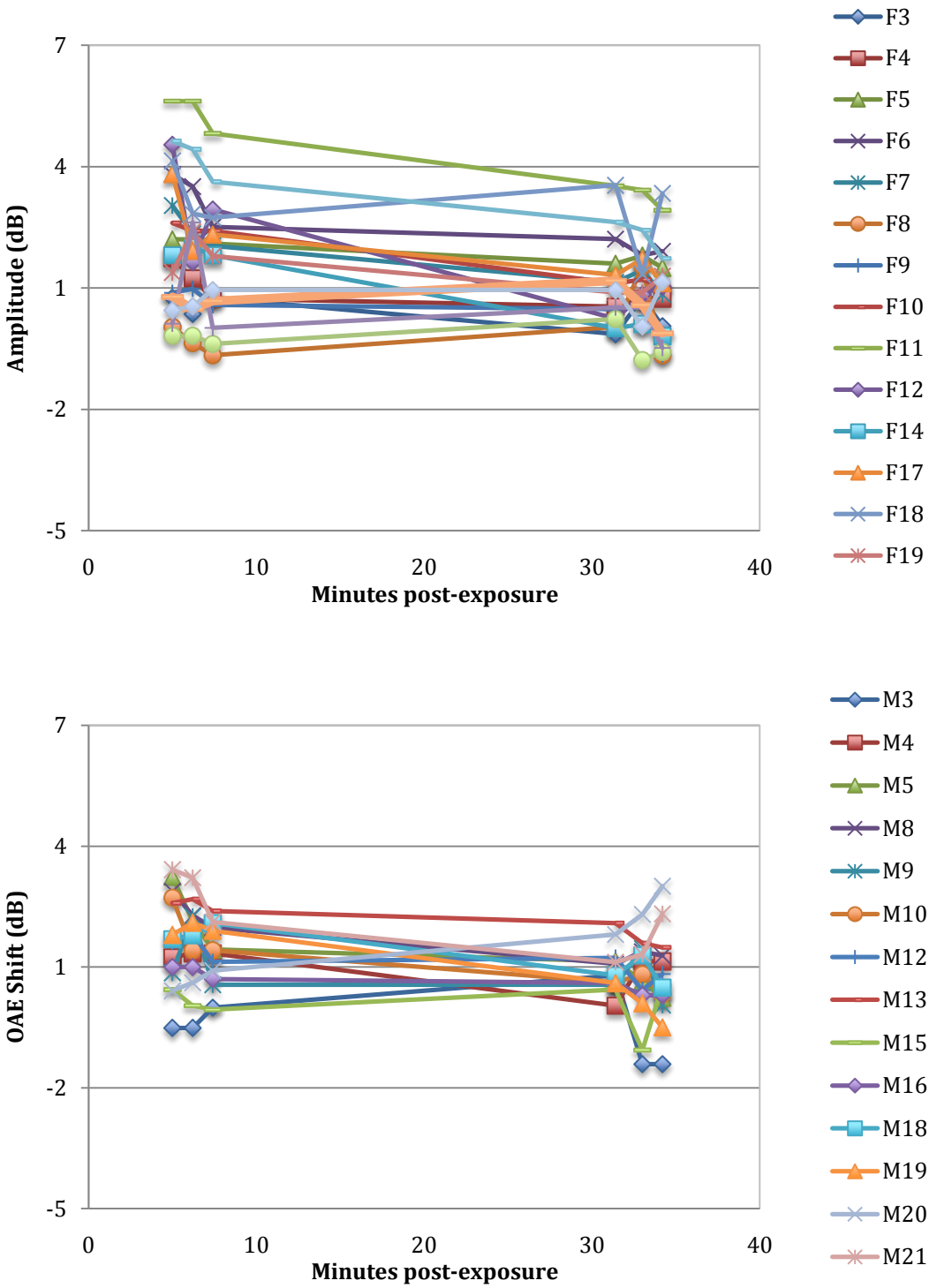


Figure 26: Individual OAE shift recovery curves for males (bottom) and females (top).

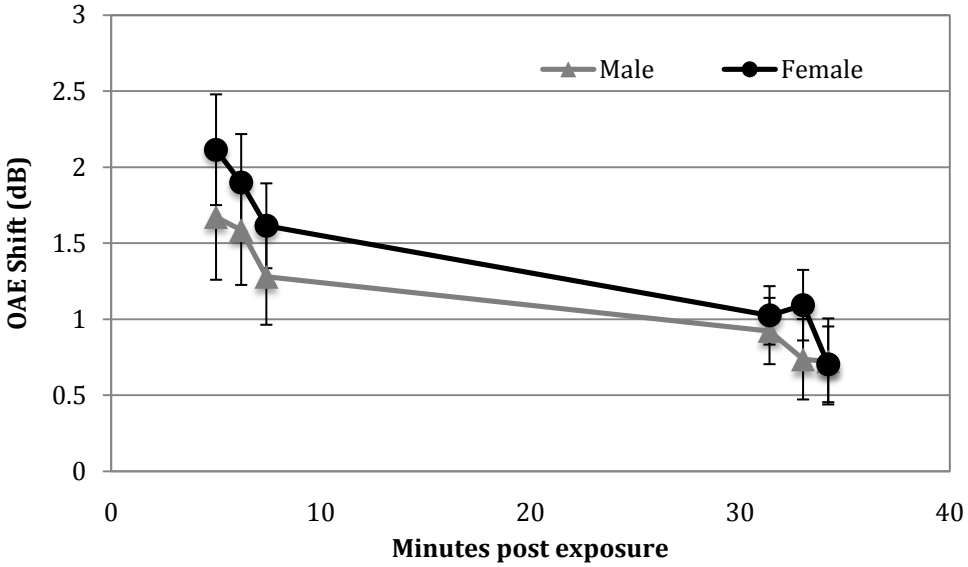


Figure 27: 4 kHz OAE shift from 5 minutes post-exposure to 34 minutes 20 seconds post exposure.

Examination of the OAE shift 1 data and OAE shift recovery data shows that there was no sex difference in either initial OAE shift or in recovery from OAE shift.

Aim 2: To determine whether there was an association between estrogen and progesterone levels and TTS and TEOAE shift in female participants and whether males, OC females and females in the early-follicular, late-follicular and luteal phases differed in TTS and TEOAE shift

Hormone group differences in TTS 1 and OAE shift 1

TTS 1

While there were no sex differences apparent in mean TTS 1 when males and the entire female group were compared (see Figure 20), a one-way ANOVA showed that TTS 1 was significantly different when hormone levels were taken into account: Welch's $F(4, 8.534) = 22.073$, $p < 0.001$. TTS 1 in the luteal group was significantly less than TTS 1 in the late-follicular group (4.41 dB ± 2.11 compared with 17.35 dB ± 1.15), and male TTS 1 (0.001) (12.47 ± 5.94) (Figure 28). There were no significant differences amongst the other groups.

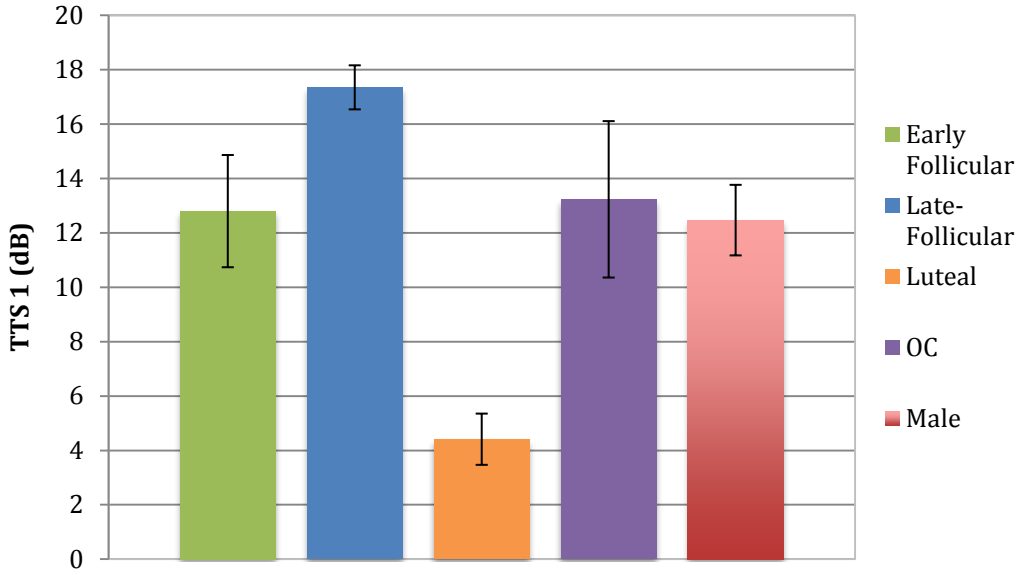


Figure 28: TTS 1 in the late follicular, early-follicular, luteal, OC and male groups. There were significant differences between the luteal group and the late-follicular and male groups.

OAE shift 1

There was a marginally significant difference in OAE shift 1 amongst these five groups: F (4, 27) = 1.850, p = 0.148. As with TTS 1 the luteal group had the smallest OAE shift 1 (0.83 dB ± 1.10) (see Figure 29).

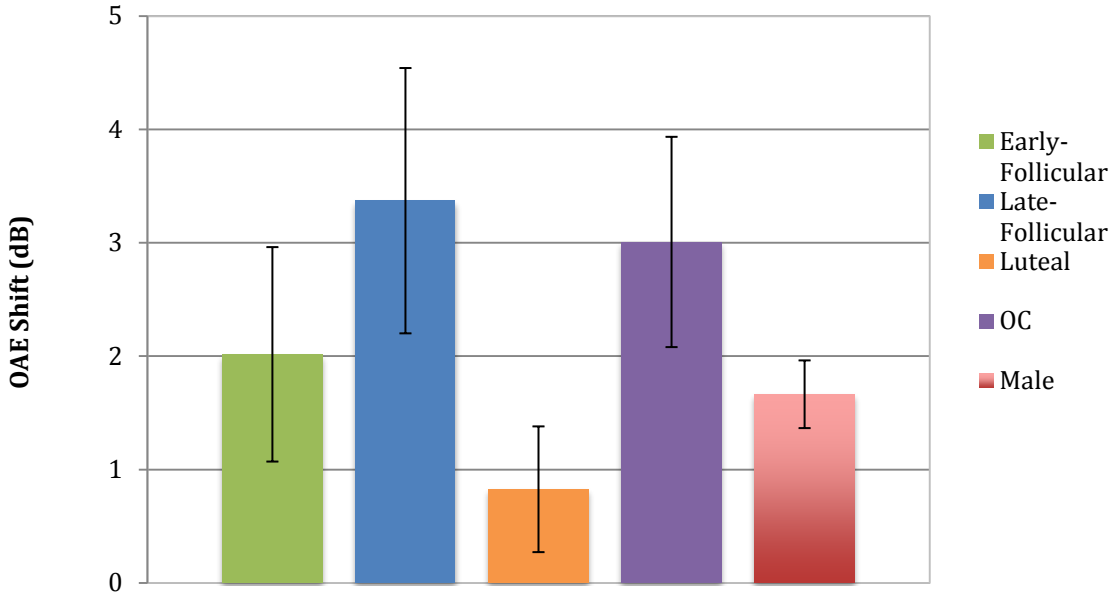


Figure 29: OAE Shift 1 in the late follicular, early-follicular, luteal, OC and male groups.

Bivariate analyses of the relationship between estrogen, progesterone, TTS and OAE shift

Estrogen

There was no significant association between estrogen levels and TTS 1: r = 0.295, p = 0.267 or OAE shift 1: r = 0.271, p = 0.371.

Progesterone

There was an association between progesterone level and TTS 1: r = 0.791, p < 0.001 and a marginal association with OAE shift 1: r = 0.538, p = 0.058. Those with low progesterone had greater TTS 1 and OAE shift 1 (Figure 30 and Figure 31).

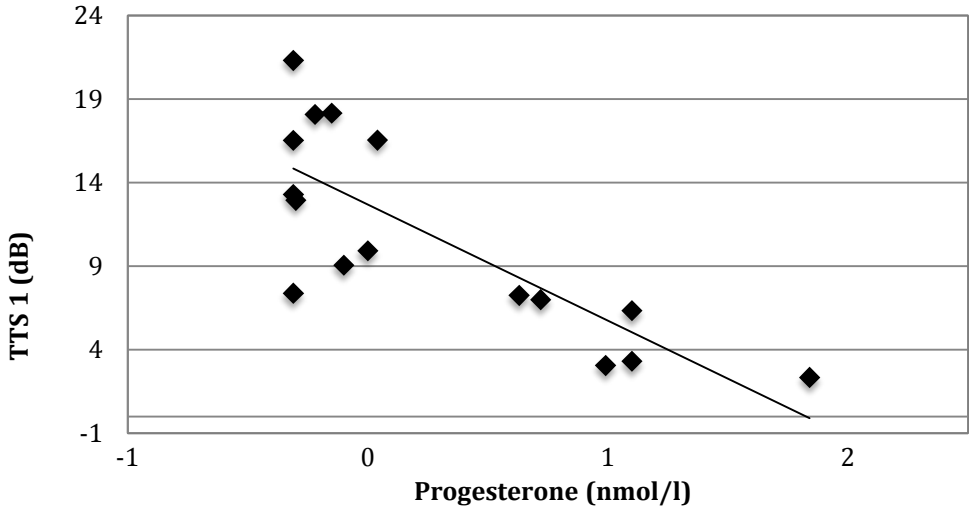


Figure 30: Scatterplot of progesterone level (nmol/l) and TTS 1. Progesterone data has been logged.

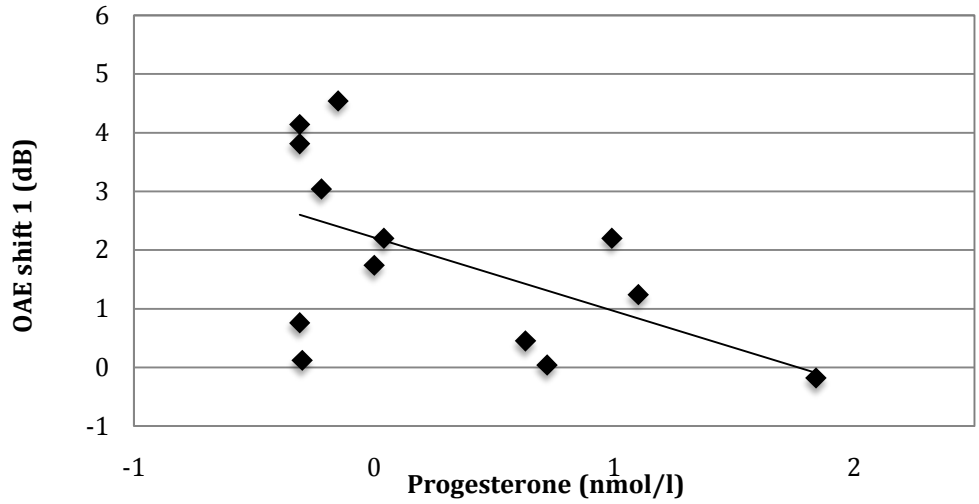


Figure 31: Scatterplot of progesterone level (nmol/l) and OAE shift 1. Progesterone data has been logged.

Hormone group differences in recovery from TTS and OAE shift

TTS recovery

TTS recovery was assessed in these five groups only at time points at which all participants had valid data points. This meant that recovery could be assessed at 12 time points between 1 minute and 30 minutes 20 seconds. This analysis showed a significant interaction between hormone group and post-exposure time on TTS: F (44, 363) = 2.580, p = 0.018 (Figure 32).

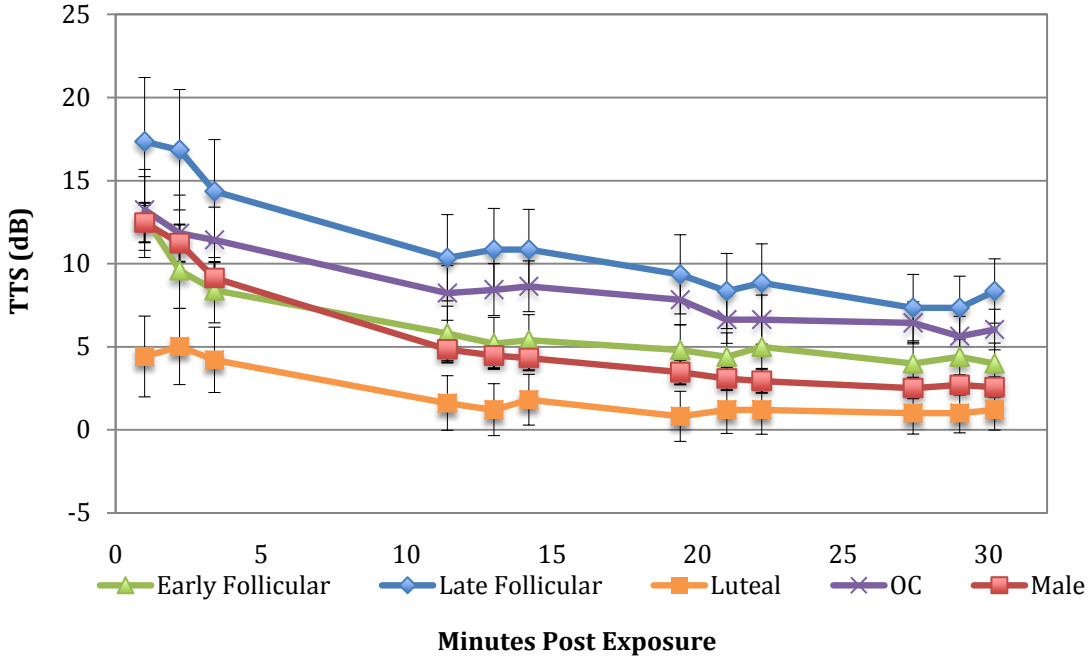


Figure 32: TTS recovery from 1 minute post exposure to 30 minutes 20 seconds post exposure.

Observation of the recovery curves in Figure 32 indicates that while the male, OC and early follicular groups had similar TTS 1, males appeared to show greater recovery from TTS: TTS 1 was 12.47 dB ± 5.94 and final TTS (30 minutes 20) was 2.56 dB ± 2.64. Early follicular TTS 1 was 12.80 dB ± 4.61 and final TTS was 3.40 dB ± 2.94. OC females had the least recovery: TTS 1 was 13.23 dB ± 6.43 and final TTS was 6.03 ± 3.77. While TTS 1 for males (12.47 dB ± 5.94) was much larger than luteal TTS 1 (4.41 dB ± 2.11), the final TTS for the two groups was quite similar: (male = 2.56 dB ± 2.64 and luteal = 1.21 dB ± 1.54).

To investigate this effect further, the percentage of TTS recovery that had occurred by 30 minutes and 20 seconds was calculated and differences were assessed with a

one-way ANOVA. The percentage of recovery was different in these five groups: Welch's F (4, 5.954) = 12.082, p = 0.005. Male percentage recovery (81.86% ± 19.99) was greater (0.001) than early-follicular recovery (27.60% ± 15.36) and OC recovery (< 0.001) (43.00% ± 9.27). There were no significant differences in the percentage of recovery amongst the female groups.

There was also a main effect of hormone group on TTS: F (4, 33) = 3.409, p = .019. There was a significant difference between the luteal group and the male group, with the male group having the larger TTS when collapsed across time: 5.313 dB ± 0.748 SEM compared with 2.060 dB ± 1.533 SEM.

OAE shift recovery

There were no differences in recovery from OAE shift amongst the hormone groups: there was no interaction of group and post-exposure time on OAE shift: F (5, 110) = 1.029, p = 0.435. There was a marginal main effect of group on OAE shift: F (4, 22) = 2.697, p = .057. This marginal effect is graphed in Figure 33 which shows that OAE shift in the luteal group was less than the other groups when collapsed across time.

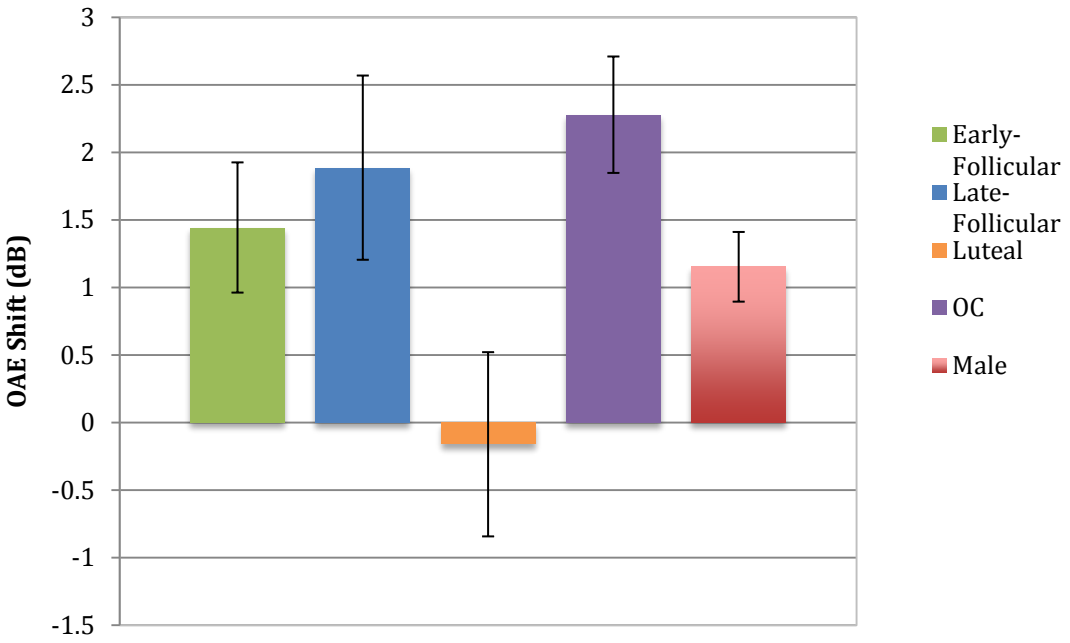


Figure 33: OAE shift collapsed across the six post-exposure measurements in the late follicular, early-follicular, luteal, OC and male groups.

Aim 3: To determine whether the associations between auditory function measures (TEOAE amplitude, efferent suppression and 4 kHz threshold) and TTS and TEOAE shift differed in males and females

The relationship of overall efferent suppression size, 4 kHz TEOAE amplitude, 4 kHz PTA threshold with the size of TTS 1 and OAE shift 1 was compared in males and females. The r and p values and N's of these associations are summarised in Table 4 on page 101.

Does efferent suppression predict TTS 1 and OAE shift 1?

The magnitude of efferent suppression for 70 dB TEOAEs and the persistence over time of efferent suppression magnitude was measured in males and females using RM ANOVA. As there was no difference in the magnitude of efferent suppression during the 6 minutes and 40 seconds of contralateral stimulation (5 measurements), no interaction between sex and the contralateral stimulus: $F(7, 217) = 0.106$, $p = 0.947$ and no main effect of sex on suppression magnitude: $F(1, 23) = 0.243$, $p = 0.626$, only the first measurement was used in correlation with TTS and OAE shift measures. This was compared with efferent suppression of 60, 65, 75 and 80 dB TEOAEs.

TTS 1

Females

There was no association between TTS 1 and efferent suppression magnitude at 60, 70, 75 or 80 dB (all $p > 0.204$). There was a marginal association with 65 dB efferent suppression magnitude and TTS 1 ($r = -0.398$, $p = 0.092$) (Figure 34).

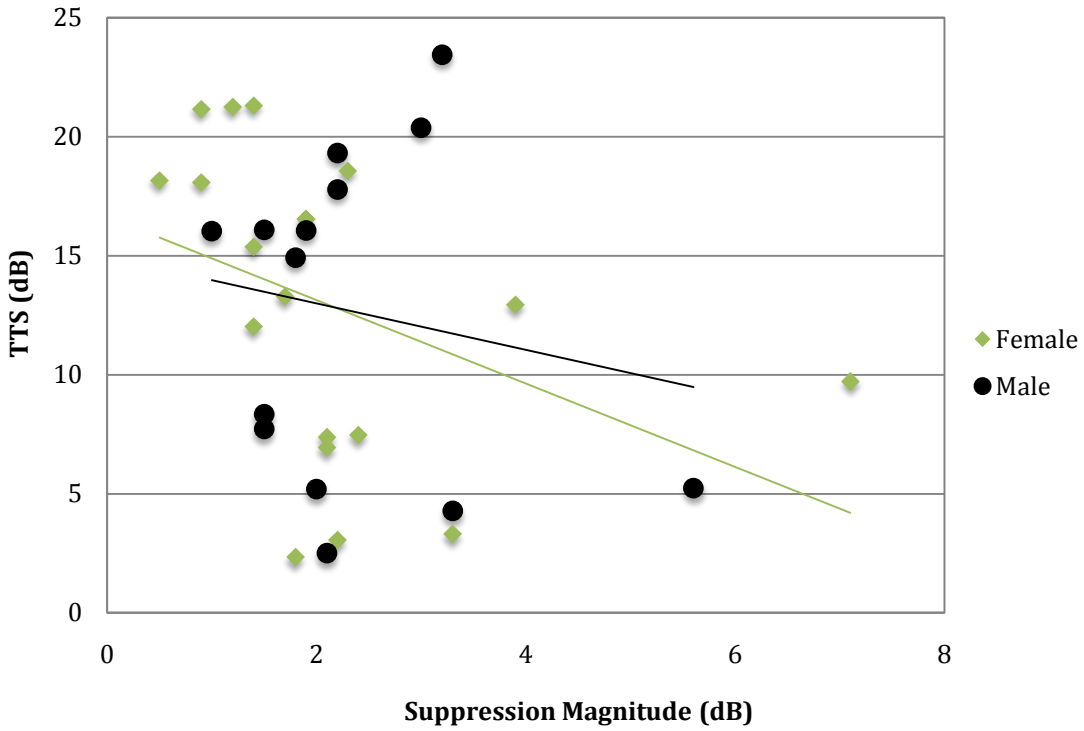


Figure 34: Scatterplot of the relationship between 65 dB efferent suppression magnitude and TTS.

Males

There was no association between TTS 1 and efferent suppression magnitude at any TEOAE stimulus level (all $p > 0.535$).

OAE shift 1

Females

There was a significant association between OAE shift 1 and efferent suppression magnitude for the two lowest TEOAE stimulus levels: 60 dB ($r = -0.505$, $p = 0.032$), 65 dB ($r = -0.513$, $p = 0.030$). Those who had high suppression amplitudes had low OAE shift (Figure 35).

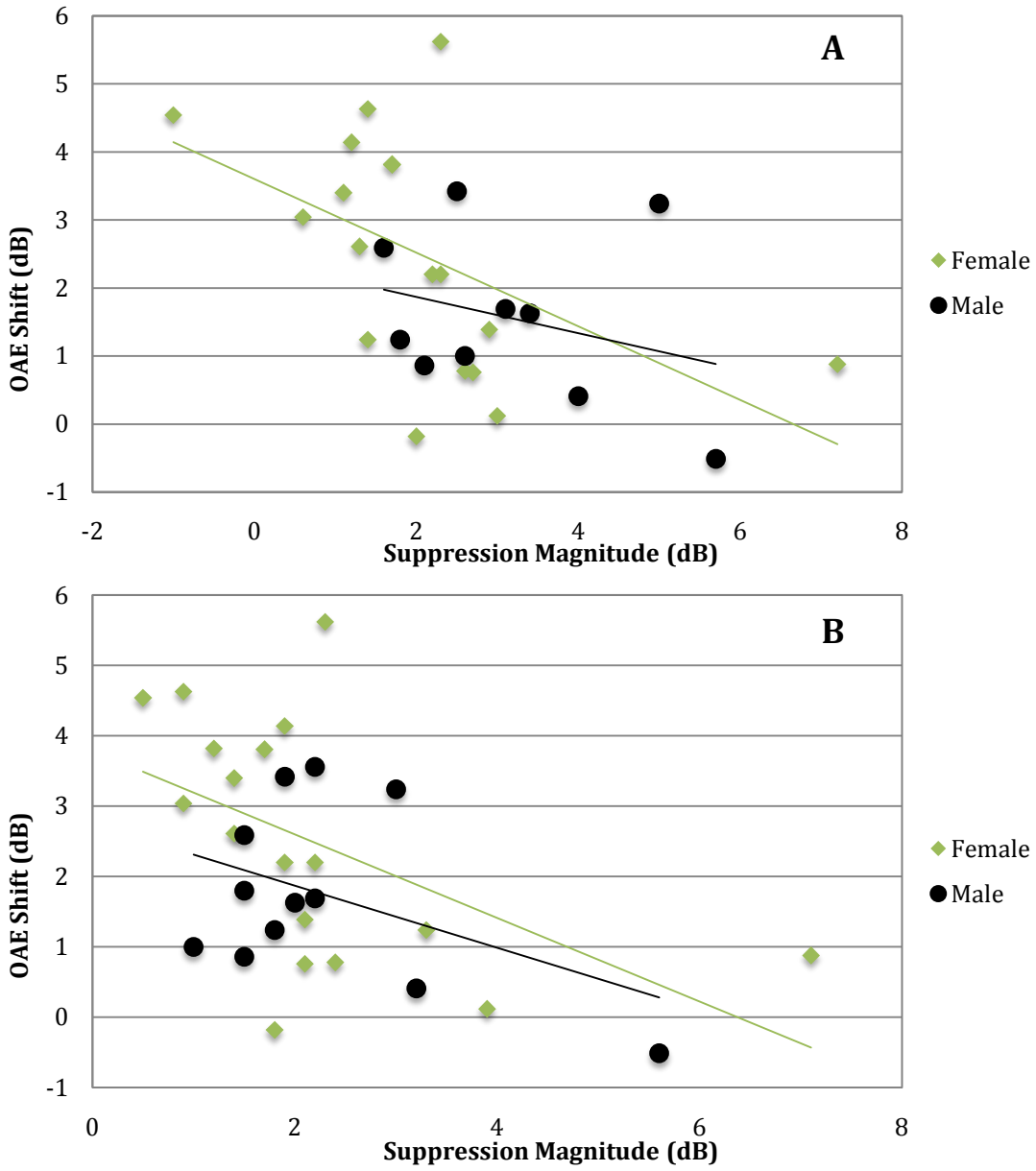


Figure 35: Scatterplots of A) 60 dB efferent suppression amplitude and OAE shift 1 and B) 65 dB efferent suppression amplitude.

There was no association between OAE shift 1 and efferent suppression magnitude at 70, 75 dB and 80 dB ($p > 0.332$).

Males

There was no association between OAE shift 1 and efferent suppression magnitude in males at any intensity level ($p > 0.161$).

Does 4 kHz OAE amplitude predict TTS 1 and OAE shift 1?

TTS 1

Female

There was no association between 4 kHz OAE amplitude and TTS 1 at 60, 65, 75 or 80 dB: ($p > .195$). There was an association at 70 dB ($r = 0.443$, $p = 0.034$). Those with larger amplitude (better) OAEs had more TTS (Figure 36).

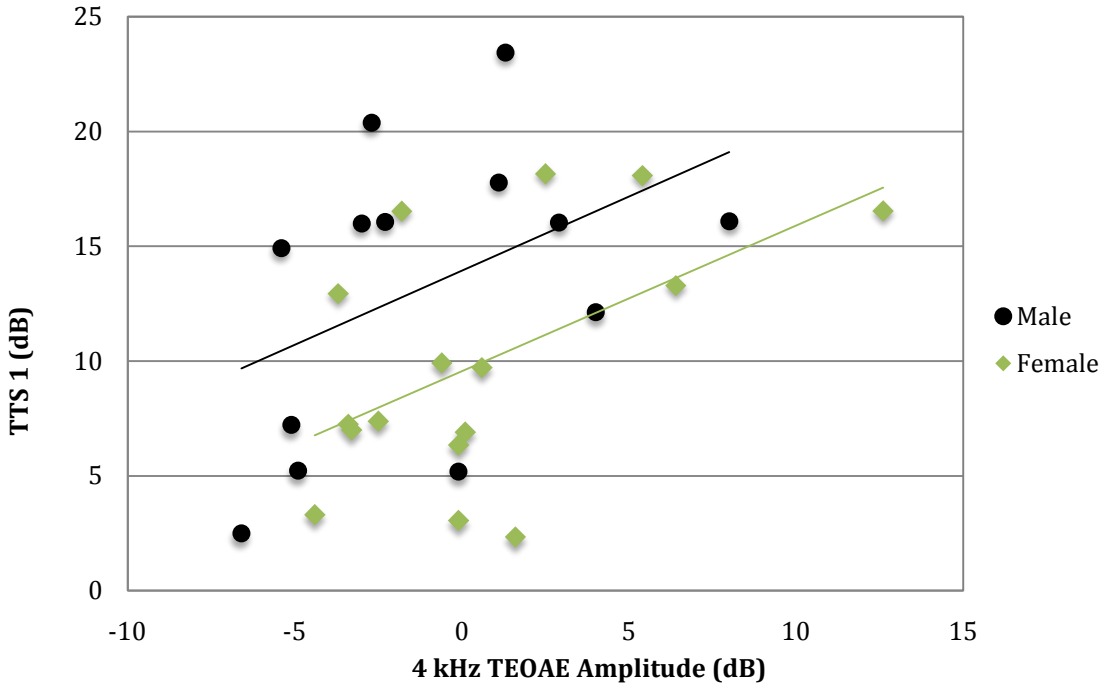


Figure 36: Scatterplot of the relationship between 4 kHz 70 dB amplitude and TTS 1.

Male

There was no association between 4 kHz OAE amplitude and TTS 1 at 60 or 65 dB ($p > 0.154$). There were marginal associations at 70 dB ($r = 0.435$, $p = 0.137$) (Figure 36), 75 dB ($r = 0.466$, $p = 0.080$) and 80 dB ($r = 0.444$, $p = .074$). Those with higher amplitude (better) OAEs had more TTS 1 (Figure 37).

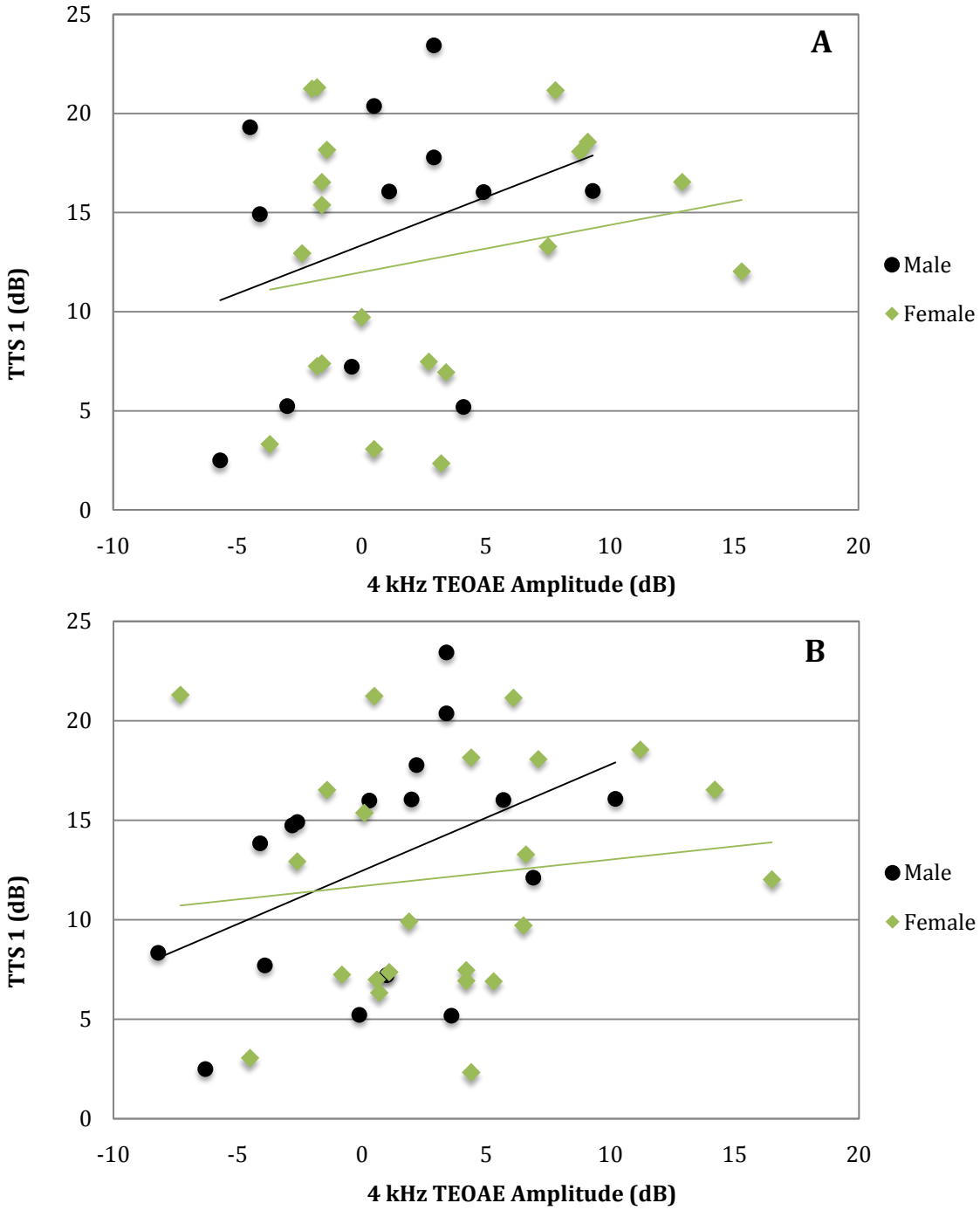


Figure 37: Scatterplot of the relationship between A) 4 kHz 75 dB and B) 4 kHz 80 dB amplitude and TTS 1.

OAE shift 1

Female

There were marginal associations between OAE shift 1 and OAE amplitude at 60 (r = 0.417, p = 0.085) and 65 dB (r = 0.397, p = 0.103) (Figure 38).

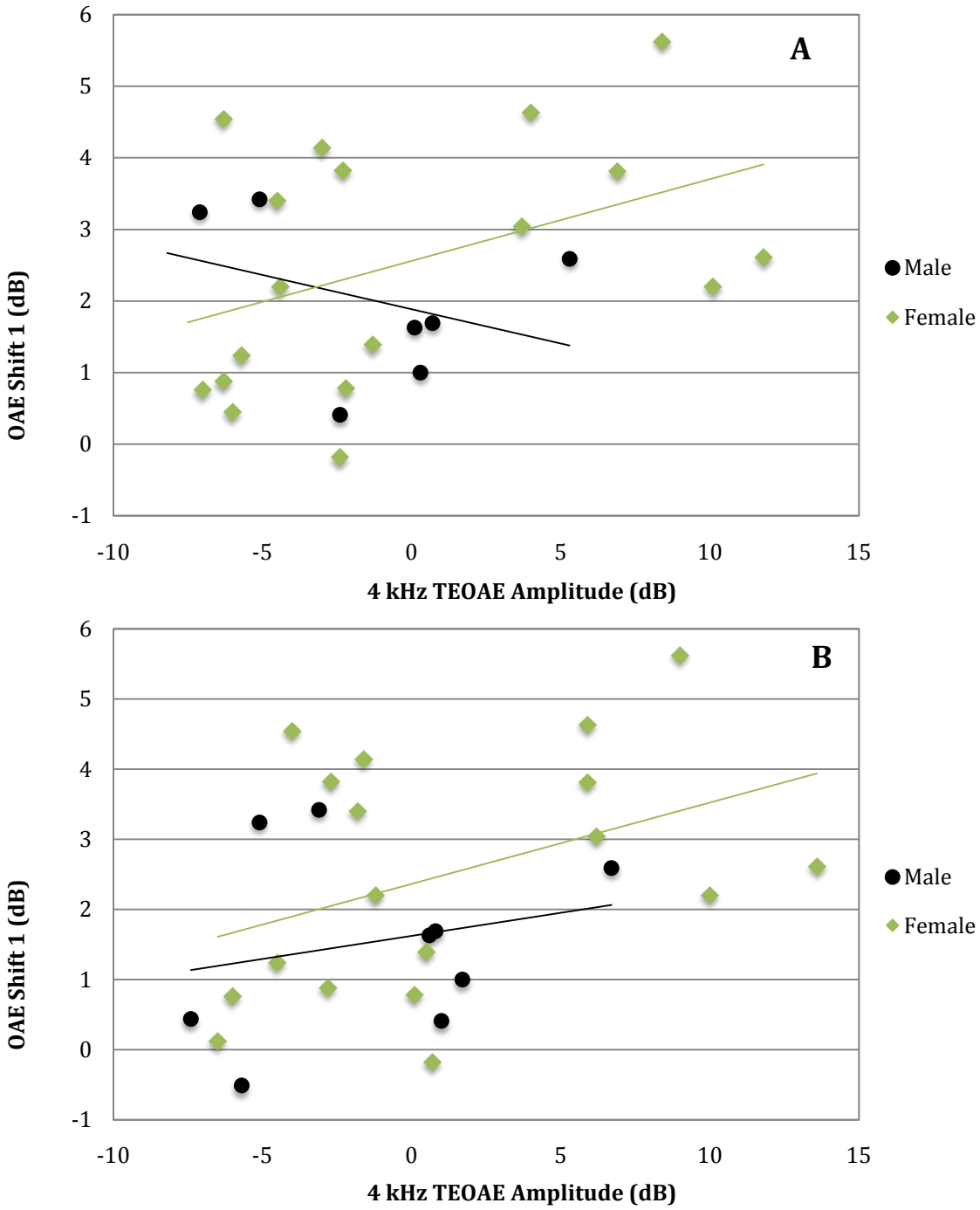


Figure 38: Scatterplot showing the relationship between A) 4 kHz 60 dB and B) 65 dB 4 kHz OAE amplitude and OAE shift 1

There was a significant association at 70 dB (0.491, p = 0.020) (Figure 39). Those with high (better) OAE amplitudes had higher OAE shift 1.

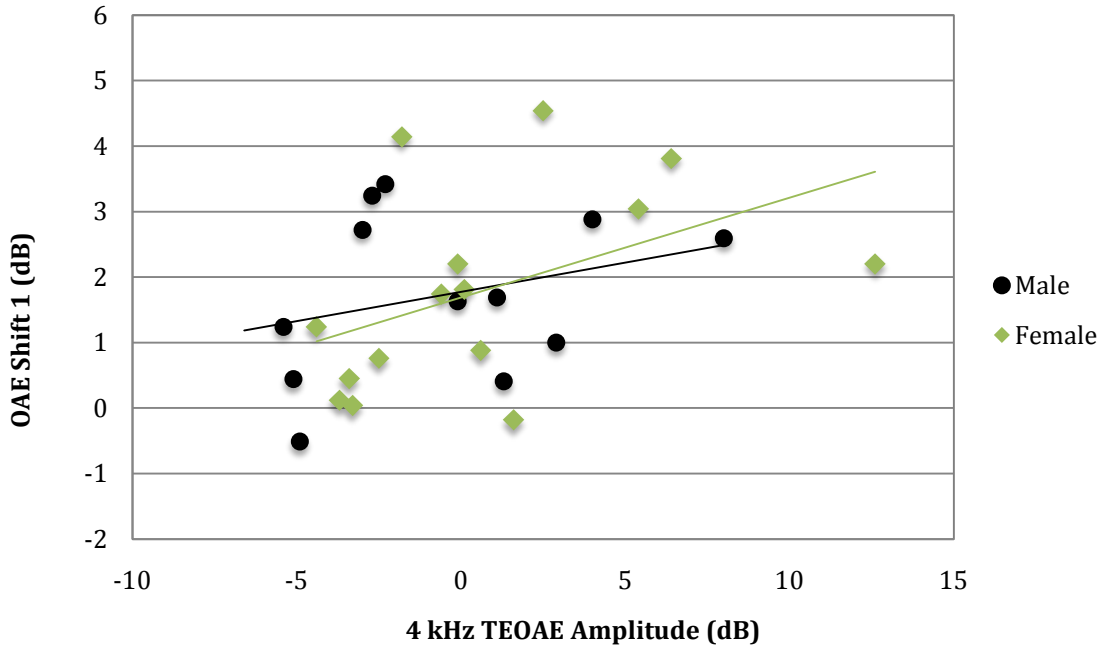


Figure 39: Scatterplot showing the relationship between 70 dB 4 kHz OAE amplitude and OAE shift 1.

There was no association at 75 or 80 dB ($p > 0.210$).

Male

There were no associations between OAE amplitude and OAE shift 1 at any level from 60-80 dB (all $p > 0.176$).

Does 4 kHz PTA threshold predict TTS 1 and OAE shift 1?

TTS 1

Female

There was no association between 4 kHz threshold and TTS 1 in the female group ($r = -0.128$, $p = 0.541$).

Male

There was an association between 4 kHz threshold and TTS 1 in the male group ($r = -0.511$, $p = 0.018$). Those with lower (better) thresholds had greater TTS 1 (Figure 40).

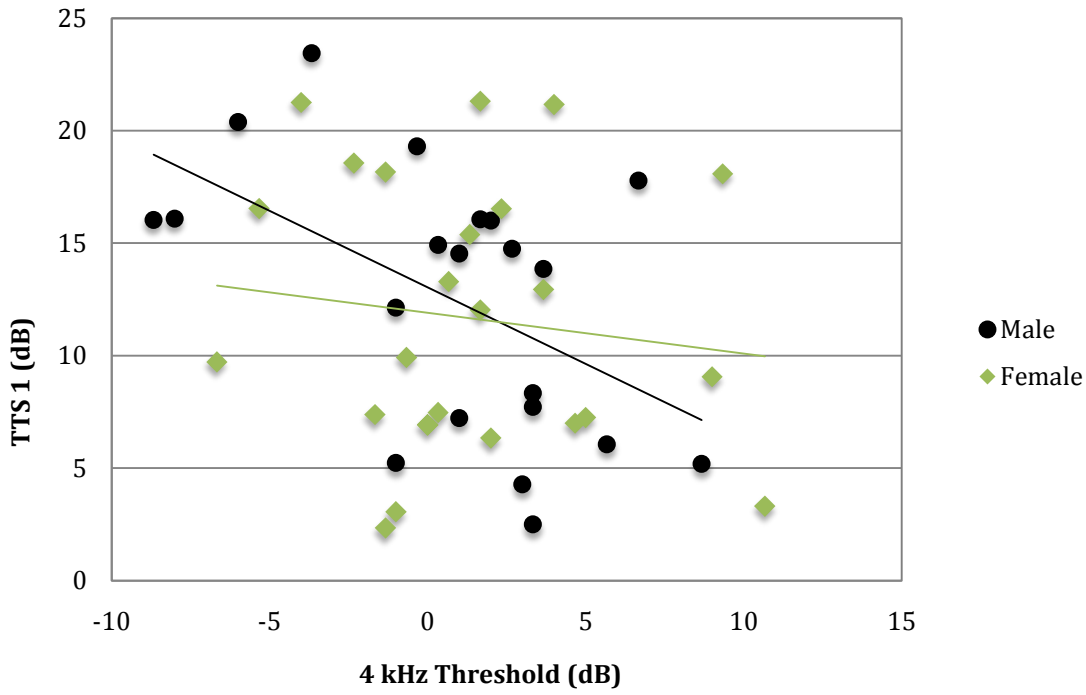


Figure 40: Scatterplot of the relationship between 4 kHz threshold and TTS 1.

OAE shift 1

Female

There was no association between 4 kHz threshold and OAE shift 1 (r = -0.103, p = 0.653).

Male

There was also no association between 4 kHz PTA threshold and OAE shift 1 (r = -.089, p = 0.734).

	Female		Male	
	TTS	OAE Shift	TTS	OAE Shift
Estrogen	r = 0.295 p = 0.267 n = 16	r = 0.271 p = 0.371 n = 13	n/a	n/a
Progesterone	r = -0.791 p < 0.001 n = 16	r = -0.538 p = 0.058 n = 13	n/a	n/a
Efferent Suppression Magnitude (Overall)				
60 dB	r = -0.305 p = 0.205 n = 19	r = -0.505 p = 0.032 n = 18	r = -0.146 p = 0.669 n = 11	r = -0.293 p = 0.411 n = 10
65 dB	r = -0.398 p = 0.092 n = 19	r = -0.513 p = 0.030 n = 18	r = -0.164 p = 0.575 n = 14	r = -0.426 p = 0.168 n = 12
70 dB	r = 0.053 p = 0.816 n = 22	r = -0.014 p = 0.954 n = 20	r = 0.034 p = 0.888 n = 20	r = 0.222 p = 0.392 n = 17
75 dB	r = -0.222 p = 0.333 n = 21	r = -0.235 p = 0.333 n = 19	r = -0.49 p = 0.857 n = 16	r = -0.208 p = 0.476 n = 14
80 dB	r = 0.230 p = 0.317 n = 21	r = -0.165 p = 0.499 n = 19	r = 0.167 p = 0.536 n = 16	r = -0.395 p = 0.162 n = 14
4 kHz OAE Amplitude				
60 dB	r = 0.305 p = 0.204 n = 19	r = 0.417 p = 0.085 n = 18	r = 0.177 p = 0.675 n = 8	r = -0.348 p = 0.445 n = 7
65 dB	r = 0.321 p = 0.195 n = 20	r = 0.397 p = 0.103 n = 18	r = 0.438 p = 0.155 n = 12	r = 0.230 p = 0.472 n = 12
70 dB	r = 0.443 p = 0.034 n = 23	r = 0.491 p = 0.020 n = 22	r = 0.435 p = 0.137 n = 13	r = 0.294 p = 0.353 n = 12
75 dB	r = 0.212 p = 0.370 n = 20	r = 0.301 p = 0.211 n = 19	r = 0.466 p = 0.080 n = 15	r = 0.129 p = 0.674 n = 13
80 dB	r = 0.126 p = 0.568 n = 23	r = 0.281 p = 0.217 n = 21	r = 0.444 p = 0.074 n = 17	r = 0.368 p = 0.177 n = 15
4 kHz Threshold				
	r = -0.128 p = 0.541 n = 25	r = -0.103 p = 0.653 n = 22	r = -0.511 p = 0.18 n = 21	r = -0.089 p = 0.734 n = 17

Table 4: Summary of the r, p and N values for the bivariate analyses between efferent suppression magnitude, TEOAE amplitude and 4 kHz threshold with TTS and OAE shift in males and females. Significant associations are highlighted in pink, marginally significant associations (< 0.150) are highlighted in blue.

Aim 4: To determine whether there was an association between estrogen and progesterone levels and auditory function measures in female participants

Efferent suppression

Estrogen

There was an association between estrogen and efferent suppression magnitude at 75 dB (r = -0.549, p = 0.042) (Figure 41).

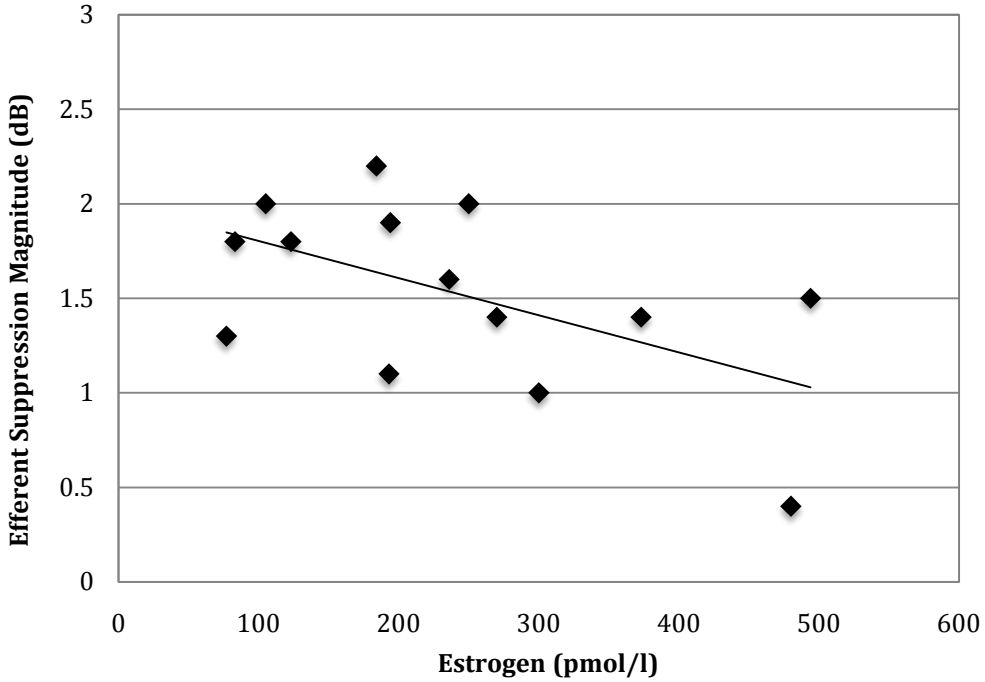


Figure 41: Scatterplot of the relationship between estrogen and 75 dB efferent suppression magnitude.

There was no association between estrogen and efferent suppression magnitude at any other TEOAE stimulus level (all p > 0.526). See Table 5 for a summary of r, p and n values of all bivariate correlations between estrogen and progesterone and the auditory predictors of TTS 1 and OAE shift 1.

Progesterone

There was also no association between progesterone and suppression magnitude at any level (all p > 0.202).

Hormone group differences in efferent suppression magnitude

There was an interaction between hormone group and TEOAE intensity on efferent suppression magnitude: F (12, 76) = 2.833, p = 0.012. There was no main effect of hormone group on suppression magnitude: F (3, 19) = 1.072, p = 0.384.

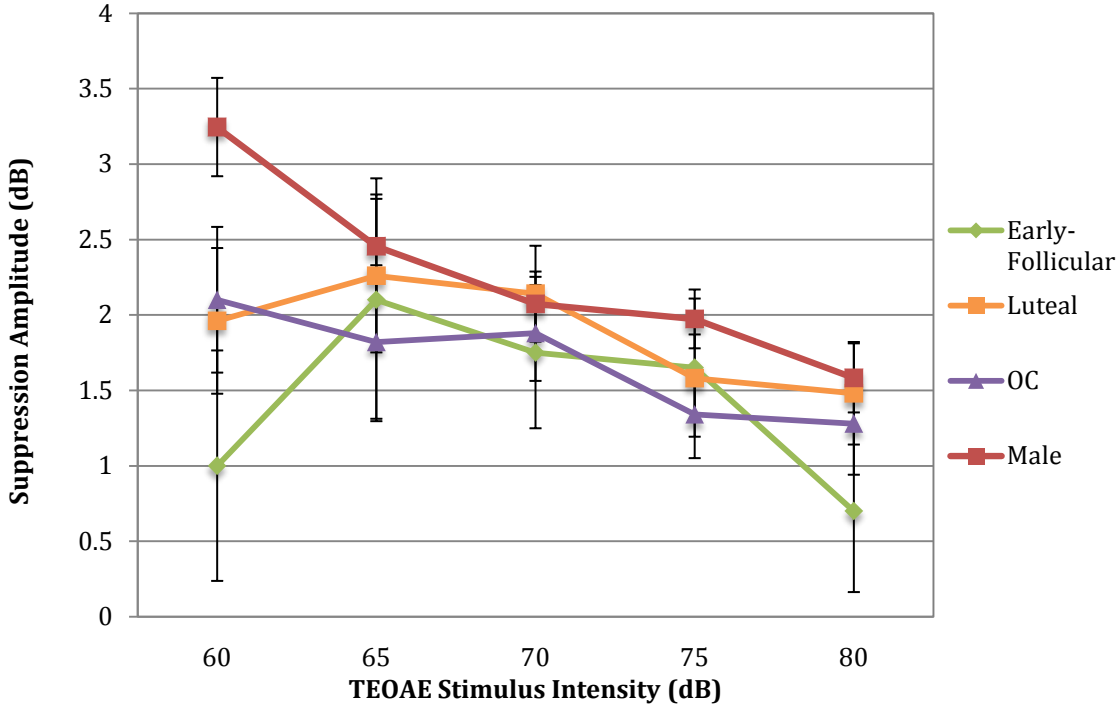


Figure 42: Efferent suppression magnitude for the different hormone groups for TEOAE stimulus intensities from 60-80 dB.

Observation of Figure 42 shows that male suppression is mostly greater than that of the female hormone groups at each intensity, however the early-follicular group shows an unusual pattern of having low suppression amplitude at 60 and 80 dB and higher amplitude suppression from 65-75 dB.

The pattern whereby male suppression values are higher than those of the female hormone groups resembles that found in the comparison between the male and whole female group (Figure 43). This showed a main effect of sex on suppression magnitude: F (1, 27) = 5.336, p = 0.029 and an interaction between sex and TEOAE stimulus level on efferent suppression magnitude: F (4, 108) = 5.294, p = 0.004 whereby male suppression is larger than female at all intensities, with only a small difference at 80 dB and the largest difference occurring at 60 dB.

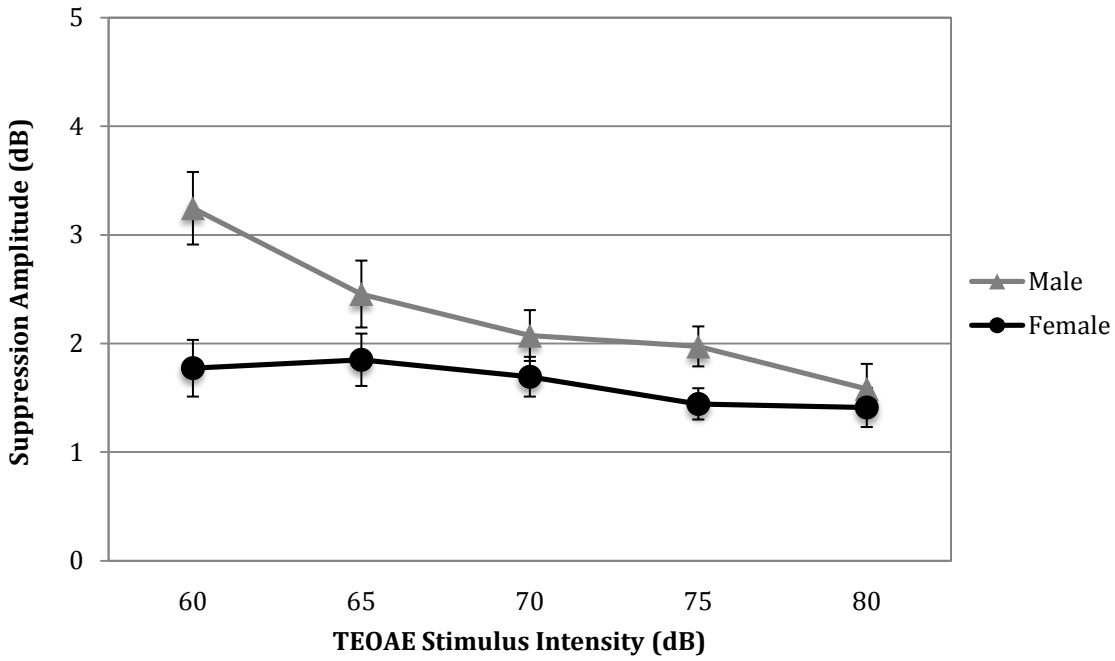
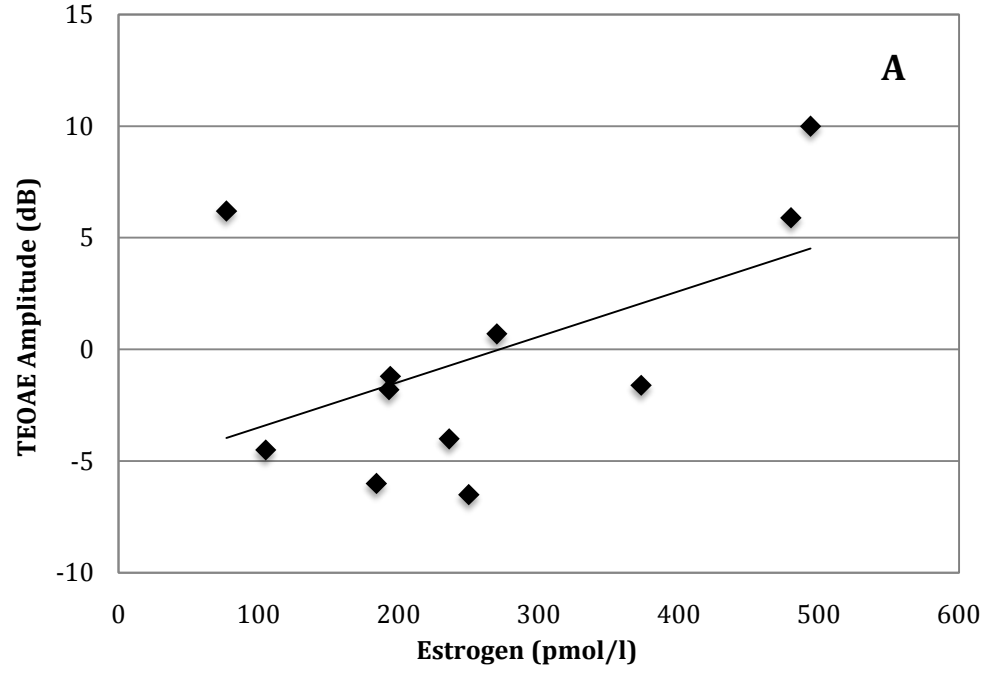


Figure 43: Efferent suppression magnitude for males and females for TEOAE stimulus intensities from 60-80 dB.

4 kHz TEOAE amplitude

Estrogen

There was a marginal association between estrogen levels and TEOAE amplitude at 65 dB (r = 0.515, p = 0.105) and at 75 dB (r = 0.492, p = 0.088) (Figure 44).



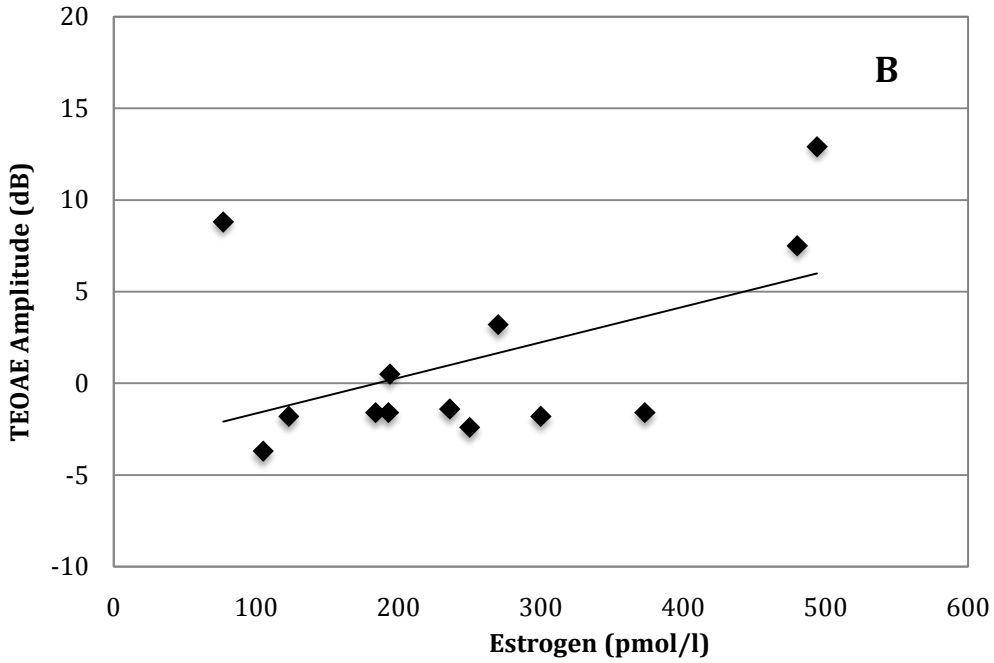
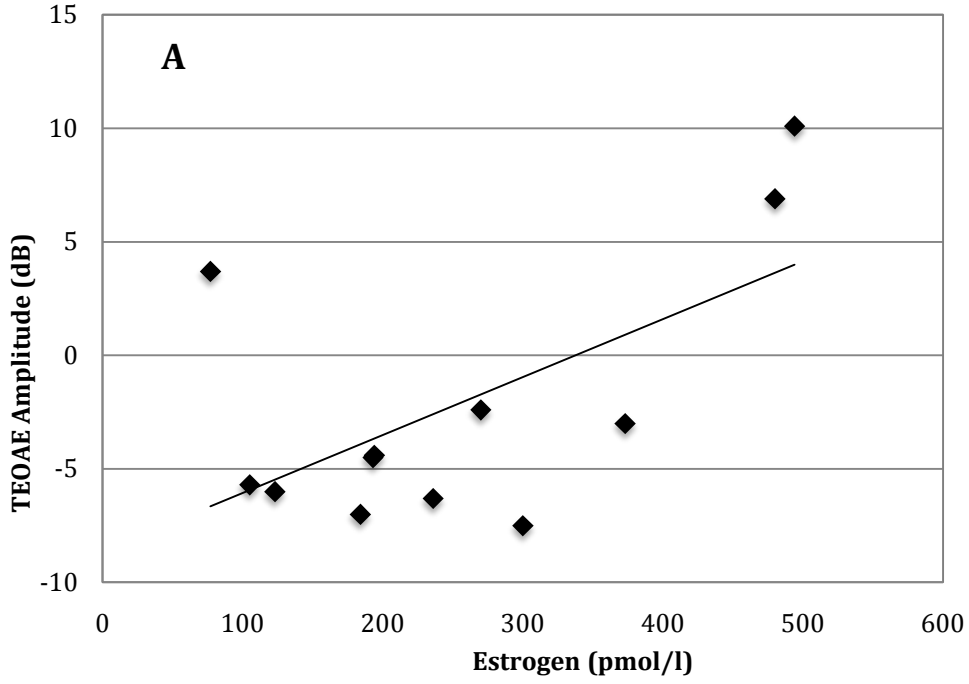


Figure 44: Scatterplot of the relationship between estrogen and A) 65 dB and B) 75 dB TEOAE amplitude.

There were significant correlations at 60 dB ($r = 0.603$, $p = 0.038$), 70 dB ($r = 0.794$, $p < 0.001$) and 80 dB ($r = 0.742$, $p = 0.002$). Those with larger estrogen levels had higher OAE amplitudes (Figure 45).



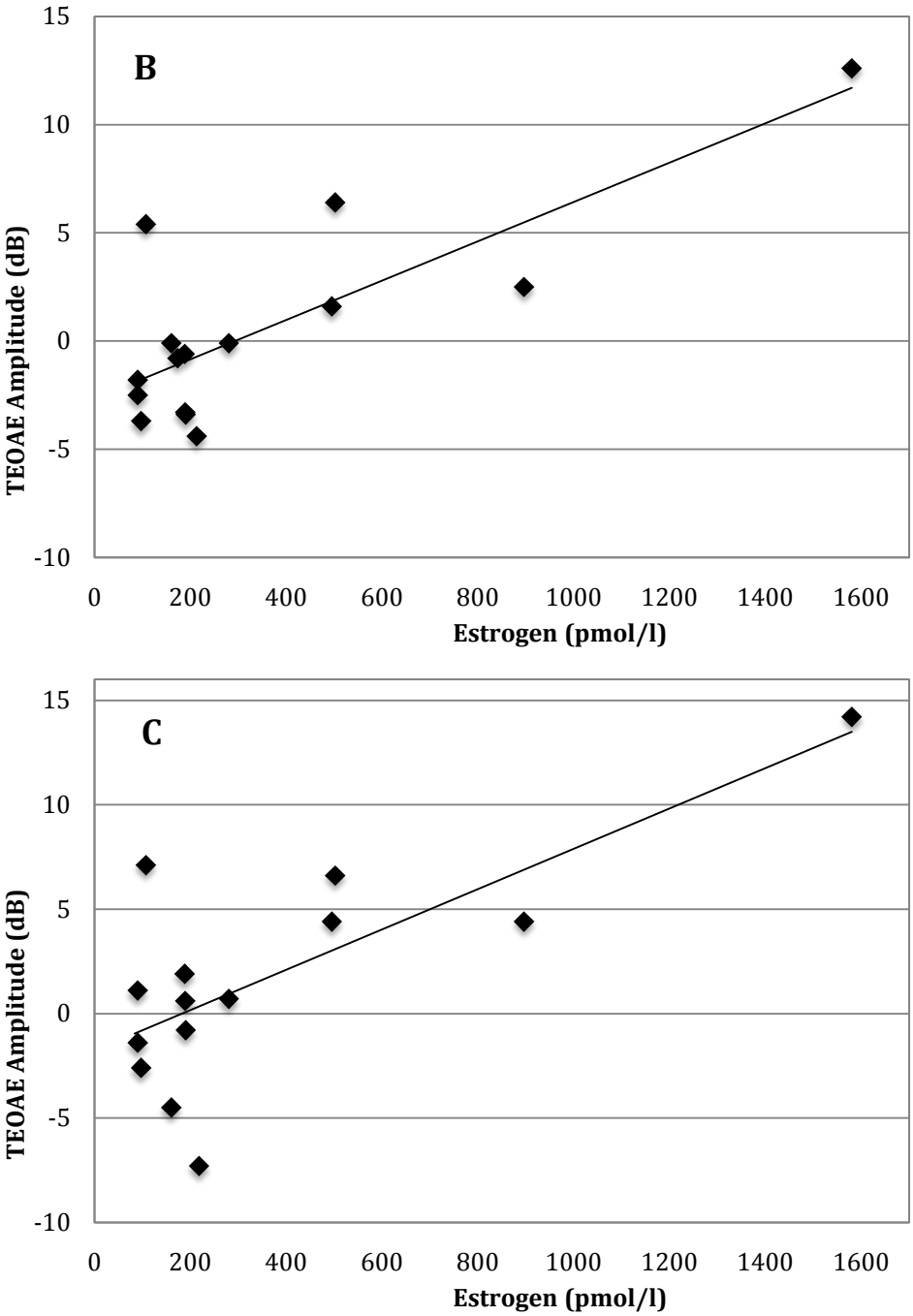


Figure 45: Scatterplot of the relationship between estrogen levels and A) 60 dB B) 70 dB and C) 80 dB 4 kHz OAE amplitude.

Progesterone

There was no association between progesterone levels and 4 kHz TEOAEs at any intensity level (all p values > 0.320).

Hormone group differences in TEOAE amplitude

There was a difference between the hormone groups in 4 kHz TEOAE amplitude: F (4, 26) = 4.578, p = 0.006 (Figure 46). The late-follicular and OC groups have the highest OAE amplitudes. The OC group was significantly different from the luteal (p = 0.041) and male groups (p = 0.040). There was no hormone group by intensity interaction on TEOAE amplitude: F (4, 26) = 1.456, p = 0.244.

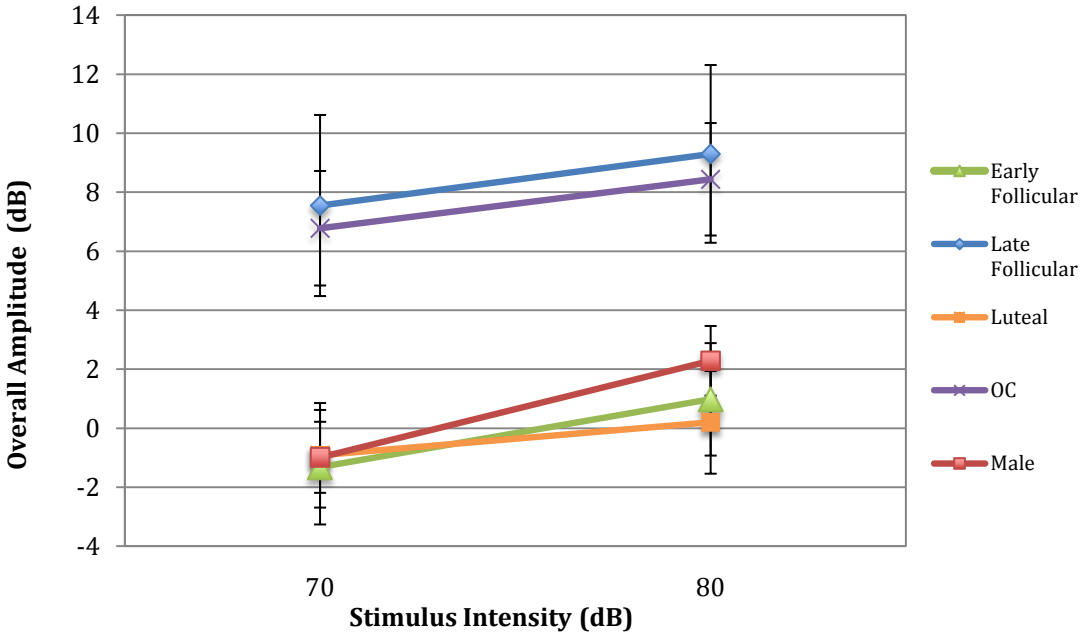


Figure 46: 4 kHz TEOAE amplitudes (70 and 80 dB) for the hormone groups.

The comparison between males and the whole female group showed a marginal interaction between sex and stimulus intensity on OAE amplitude at 4 kHz (65-80 dB): F (3, 84) = 2.061, p = 0.150 and no main effect of sex on OAE amplitude: F (1, 28) = 0.431, p = 0.517. However, the difference between male and female OAE values was largest at the lowest stimulus level and smallest at the highest level.

4 kHz threshold

Estrogen

There was also an association between estrogen levels and 4 kHz PTA threshold: (r = -0.591, p = 0.016). Those with larger estrogen levels had lower (better) thresholds (Figure 47).

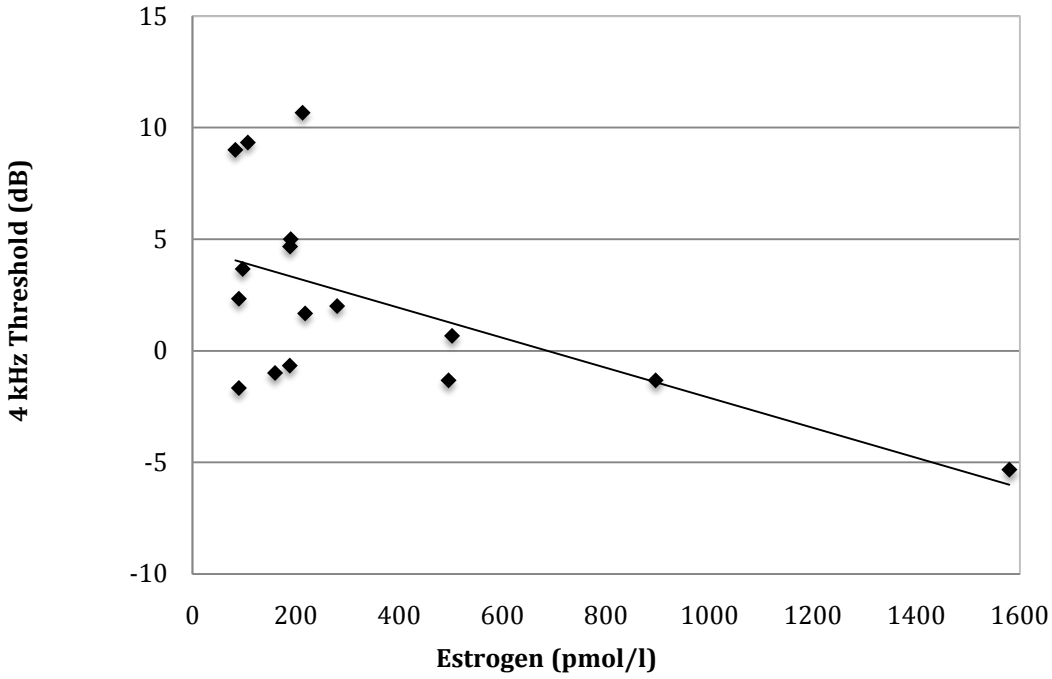


Figure 47: Scatterplot of the relationship between estrogen levels and 4 kHz threshold.

Progesterone

There was no relationship with 4 kHz threshold ($r = 0.035$, $p = 0.897$).

Hormone group differences in 4 kHz threshold

There was no difference in the mean 4 kHz threshold between the hormone groups $F(4, 33) = 1.553$, $p = 0.210$.

	Estrogen	Progesterone
Efferent Suppression Magnitude		
60 dB	r = 0.203 p = 0.527 n = 12	r = 0.309 p = 0.329 n = 12
65 dB	r = -0.065 p = 0.842 n = 12	r = 0.395 p = 0.203 n = 12
70 dB	r = 0.073 p = 0.804 n = 14	r = -0.251 p = 0.386 n = 14
75 dB	r = -0.549 p = 0.042 n = 14	r = 0.106 p = 0.717 n = 14
80 dB	r =- 0.021 p = 0.943 n = 14	r = -0.031 p = 0.916 n = 14
4 kHz OAE Amplitude		
60 dB	r = 0.603 p = 0.038 n = 12	r = 0.314 p = 0.321 n = 12
65 dB	r = 0.515 p = 0.105 n = 11	r = 0.072 p = 0.833 n = 11
70 dB	r = 0.794 p = < 0.001 n = 15	r = -0.244 p = 0.381 n = 15
75 dB	r = 0.492 p = 0.088 n = 13	r = 0.106 p = 0.731 n = 13
80 dB	r = 0.742 p = 0.002 n = 14	r = -0.045 p = 0.880 n = 14
4 kHz Threshold		
4 kHz Threshold	r = -0.591 p = 0.016 n = 16	r = 0.035 p = 0.897 n = 16

Table 5: Summary of the r, p and N values for the bivariate analyses between estrogen, progesterone and efferent suppression magnitude, TEOAE amplitude and 4 kHz threshold. Significant associations are highlighted in pink, marginally significant associations (< 0.150) are highlighted in blue.

Aim 5: To determine whether estrogen and progesterone effected susceptibility to TTS and TEOAE shift through their effects on the auditory function measures or whether they acted independently to effect TTS and TEOAE shift

To assess the mediating role of efferent suppression magnitude, TEOAE amplitude and 4 kHz threshold on the relationship between hormones TTS and OAE shift, linear regression models were produced. A separate model was produced to assess the mediating role of each auditory function measure on TTS and on OAE shift, so each model includes all those participants who had data available for that auditory

function measure and either TTS or OAE shift data, meaning that participant numbers differed slightly between models. Each model had three levels: the first level looked at the effect of estrogen alone on TTS and OAE shift; the second looked at the effect of estrogen and progesterone together on TTS and OAE shift; and the third looked at the effect of estrogen and progesterone as well as the auditory measure on TTS and OAE shift. These models are summarised in Table 6.

It can be seen from Table 6 that estrogen alone only explained a small (non-significant) part of the variance of TTS ($r = 0.295$, $p = 0.267$) and of OAE shift ($r = 0.271$, $p = 0.371$). The small relationship was positive, with higher estrogen associated with more TTS and OAE shift. When progesterone was added to the model it explained a larger part of the variance in TTS ($r = -0.789$, $p < 0.001$) and OAE shift ($r = -0.528$, $p = 0.065$) than estrogen alone. There was a larger association with TTS than with OAE shift. Higher progesterone was associated with less TTS and OAE shift. The effects of estrogen and progesterone on TTS and OAE shift were independent; adding progesterone to the model did not substantially change the r-value of estrogen, (TTS (0.295 to 0.290) and OAE shift (0.270 to 0.249)). Together estrogen and progesterone accounted for 66.5% of the variance in TTS compared to 2% for estrogen alone. Estrogen and progesterone together accounted for 22.2% of the variance in OAE shift compared to 1.1% for estrogen alone.

Although there were a slightly different set of participants in each analysis the same patterns applied for each of the individual analyses. Estrogen explained a similar amount of variance in TTS and OAE shift in each analysis (small, positive, non-significant). Progesterone explained more of the variance than estrogen in both TTS and OAE shift. This was a large mostly significant relationship. Estrogen and progesterone together explained more of the variance in TTS than in OAE shift (driven by the large association of progesterone) in each of the models.

TTS

The addition of any of the auditory measures to the models did not greatly alter the amount of variance in TTS that was explained by estrogen, as reflected by few

large changes to the estrogen r-values. This suggests that the effects of estrogen and auditory function on TTS were for the most part independent apart from some small changes in the r-values of estrogen when 65, 70 and 80 dB OAE amplitude were added to the model. OAE size had a small mediating role on the effect of estrogen on TTS; once the effect of OAE size (65 dB) was added to the model, the small positive relationship of estrogen on TTS was reduced (0.328 to 0.218). The same thing occurred for 70 dB (0.329 to 0.139). After the variability due to OAE size (65 and 70 dB) was accounted for the correlation between estrogen and TTS reduced. When 80 dB OAE amplitude was added to the model the estrogen r value increased from 0.270 to 0.406.

No difference was seen in the r-values of progesterone when any of the auditory measures was added to the model, indicating that the effects of progesterone on TTS was independent of the auditory measures.

Additionally, there was almost no difference in the total variance in TTS explained by adding any auditory measure to the model as demonstrated by the lack of change in the adjusted r^2 value when the auditory measures were added to the models. This implies that auditory function did not influence TTS beyond the influence of hormone levels.

OAE shift

The addition of the auditory measures to the models had a greater impact on the amount of variance in OAE shift that was explained by estrogen than occurred in the TTS models, as reflected by the greater numbers of large changes in the estrogen r-values.

There were some small changes in the r-values of estrogen when efferent suppression at 60, 65 and 70 dB and OAE amplitude at 65 and 70 were added to the model. The effects of estrogen and other measured aspects of auditory function on OAE shift were independent.

Efferent suppression had a small mediating role between estrogen level and OAE shift; the small positive influence of estrogen on OAE shift was reduced (60 dB: 0.201 to 0.070; 65 dB: 0.201 to 0.004 and at 75 dB: 0.223 to 0.111). OAE level also had a small mediating role between estrogen level and OAE shift; the small positive influence of estrogen on OAE shift was reduced (65 dB: 0.201 to 0.059 and 70 dB: 0.249 to -0.095).

No difference was seen in the predictive power of progesterone when any of the auditory measures was added to the model, indicating that the effects of progesterone and the auditory measures on OAE shift was independent.

There was a increase in the total variance in OAE shift explained when adding many of the auditory measures to the models, suggesting that the effects of these auditory measures and hormones are independent. When efferent suppression at 80 dB and OAE amplitude at 60 and 75 dB were added to the model, the total variance explained in OAE shift decreased suggesting a mediating role of these factors on the effects of hormones on OAE shift.

In summary, there was a mediating role of some aspects of auditory function on the effects of estrogen on TTS and to a greater degree on OAE shift. However, estrogen itself only had a very small impact on TTS and OAE shift so this suggests that for the most part the impact of auditory function and hormones on OAE shift are independent.

The role of estrogen and progesterone on OAE shift						The role of estrogen and progesterone on TTS					
			r	sig	Adj r square				r	sig	Adj r square
	E only	E	0.271	0.371	-0.011		E only	E	0.295	0.267	0.022
	E and P	E	0.249	0.352	0.222		E and P	E	0.29	0.074	0.665
		P	-0.528	0.065				P	-0.789	<0.001	
Mediating Role of the Efferent System						Mediating Role of the Efferent System					
60 dB	E only	E	0.206	0.569	-0.077	60 dB	E only	E	0.239	0.478	-0.047
	E and P	E	0.201	0.547	0.095		E and P	E	0.255	0.216	0.639
		P	-0.504	0.156				P	-0.809	0.003	
	E, P and Eff 60	E	0.07	0.769	0.547		E, P and Eff 60	E	0.208	0.317	0.645
		P	-0.412	0.119				P	-0.791	0.004	
		Eff 60	-0.654	0.03				Eff 60	-0.207	0.323	
65 dB	E only	E	0.206	0.569	-0.077	65 dB	E only	E	0.239	0.478	-0.047
	E and P	E	0.201	0.547	0.095		E and P	E	0.255	0.216	0.639
		P	-0.504	0.156				P	-0.809	0.003	
	E, P and Eff 65	E	0.004	0.99	0.391		E, P and Eff 65	E	0.168	0.396	0.688
		P	-0.405	0.177				P	-0.748	0.004	
		Eff 65	-0.589	0.08				Eff 65	-0.285	0.177	
70 dB	E only	E	0.295	0.378	-0.014	70 dB	E only	E	0.348	0.244	0.041
	E and P	E	0.208	0.473	0.257		E and P	E	0.239	0.095	0.802
		P	-0.571	0.072				P	-0.852	<0.001	
	E, P and Eff 70	E	0.211	0.489	0.188		E, P and Eff 70	E	0.238	0.114	0.782
		P	-0.625	0.079				P	-0.842	<0.001	
		Eff 70	-0.169	-0.56				Eff 70	0.044	0.756	
75 dB	E only	E	0.24	0.477	-0.047	75 dB	E only	E	0.276	0.362	-0.008
	E and P	E	0.223	0.466	0.155		E and P	E	0.291	0.123	0.641
		P	-0.516	0.114				P	-0.79	<0.001	
	E, P and Eff 75	E	0.111	0.704	0.276		E, P and Eff 75	E	0.226	0.206	0.693
		P	-0.436	0.0156				P	-0.724	0.002	
		Eff 75	-0.435	0.17				Eff 75	-0.279	0.133	
80 dB	E only	E	0.24	0.477	-0.047	80 dB	E only	E	0.276	0.362	-0.008
	E and P	E	0.223	0.466	0.155		E and P	E	0.291	0.123	0.641
		P	-0.516	0.114				P	-0.79	<0.001	
	E, P and Eff 80	E	0.241	0.466	0.051		E, P and Eff 80	E	0.337	0.086	0.653
		P	-0.494	0.16				P	-0.733	0.003	
		Eff 80	-0.112	0.735				Eff 80	-0.212	0.273	
Mediating Role of TEOAE Amplitude						Mediating Role of TEOAE Amplitude					
60 dB	E only	E	0.175	0.629	-0.091	60 dB	E only	E	0.279	0.406	-0.025
	E and P	E	0.111	0.695	0.344		E and P	E	0.236	0.238	0.659
		P	-0.681	0.04				P	-0.807	0.002	
	E, P, OAE 60	E	0.097	0.796	0.235		E, P, OAE 60	E	0.256	0.328	0.612
		P	-0.674	0.071				P	-0.813	0.005	
		OAE 60	0.026	0.948				OAE 60	-0.036	0.89	
65 dB	E only	E	0.206	0.569	-0.077	65 dB	E only	E	0.336	0.342	0.002
	E and P	E	0.201	0.547	0.095		E and P	E	0.328	0.118	0.693
		P	-0.504	0.156				P	-0.805	0.003	
	E, P, OAE 65	E	0.059	0.889	0.006		E, P, OAE 65	E	0.218	0.379	0.68
		P	-0.489	0.192				P	-0.794	0.006	
		OAE 65	0.249	0.561				OAE 65	0.194	0.432	
70 dB	E only	E	0.271	0.371	-0.011	70 dB	E only	E	0.374	0.188	0.068
	E and P	E	0.249	0.352	0.222		E and P	E	0.329	0.043	0.732
		P	-0.528	0.065				P	-0.797	<0.001	
	E, P, OAE 70	E	-0.095	0.831	0.221		E, P, OAE 70	E	0.139	0.578	0.731
		P	-0.441	0.136				P	-0.751	0.001	
		OAE 70	0.438	0.346				OAE 70	0.244	0.348	
75 dB	E only	E	0.24	0.477	-0.047	75 dB	E only	E	0.261	0.413	-0.025
	E and P	E	0.223	0.466	0.155		E and P	E	0.256	0.178	0.661
		P	-0.516	0.114				P	-0.809	0.001	
	E, P, OAE 75	E	0.189	0.646	0.037		E, P, OAE 75	E	0.228	0.365	0.621
		P	-0.509	0.15				P	-0.805	0.003	
		OAE 75	0.056	0.892				OAE 75	0.047	0.85	
80 dB	E only	E	0.262	0.411	-0.025	80 dB	E only	E	0.272	0.347	-0.003
	E and P	E	0.26	0.357	0.211		E and P	E	0.27	0.131	0.644
		P	-0.535	0.077				P	-0.79	0.001	
	E, P, OAE 80	E	0.302	0.544	0.144		E, P, OAE 80	E	0.406	0.139	0.628
		P	-0.543	0.101				P	-0.798	0.001	
		OAE 80	-0.054	0.914				OAE 80	-0.183	0.486	
Mediating Role of 4 kHz Threshold						Mediating Role of 4 kHz Threshold					
4 kHz Thold	E only	E	0.271	0.371	-0.011	4 kHz Thold	E only	E	0.295	0.267	0.022
	E and P	E	0.249	0.352	0.222		E and P	E	0.29	0.074	0.665
		P	-0.528	0.065				P	-0.729	<0.001	
	E, P, thold.	E	0.31	0.369	0.145		E, P, thold.	E	0.368	0.076	0.651
		P	-0.537	0.077				P	-0.793	<0.001	
		Thold	0.106	0.756				Thold	0.132	0.5	

Table 6: Summary of data from the linear regression models to assess the role of auditory measures on mediating the relationship between hormones on TTS and OAE shift. Each model had three levels: the first was the effect of estrogen alone on TTS and OAE shift. The second looked at the effect of estrogen and progesterone together on TTS and OAE shift and the third looked at the effect of estrogen and progesterone as well as the auditory measure on TTS and OAE shift.

Discussion

Summary of results

The main objective of this study was to determine whether there were sex differences in TTS and OAE shift and to determine what effect estrogen and progesterone levels had on sex differences in human TTS and OAE shift. Additionally, it aimed to determine whether aspects of cochlear function and hearing which have been shown to be associated with susceptibility to NIHL such as OAE amplitude, efferent suppression magnitude and PTA thresholds (2-4, 27-34) are also associated with female sex hormones (3, 4, 48-50). It was also determined whether the effect of sex hormones on susceptibility to NIHL is mediated by their effect on auditory function measures or whether hormones have an independent effect on susceptibility to NIHL.

The first aim of the study was to determine whether males and females differed in TTS and TEOAE shift and recovery from TTS and TEOAE shift after exposure to a 3kHz pure-tone, 100 dB L_{Aeq} 15 minute, noise exposure. The results showed that males and the entire group of females did not differ in TTS 1, OAE shift 1 or in recovery from OAE shift. In contrast, there was a sex difference in recovery from TTS as females showed a slower recovery from TTS than males.

The second aim was to determine whether there was an association between estrogen and progesterone levels and TTS and TEOAE shift in female participants and whether males, OC females and females in the early-follicular, late-follicular and luteal phases differed in TTS and TEOAE shift. When female hormonal status was taken into account there were differences seen in TTS between males and females in the luteal phase during which progesterone levels are highest, with luteal females having less TTS. The same pattern was observed in the OAE shift data. This was highlighted by the presence of a large negative correlation between progesterone and TTS and OAE shift, which was larger and significant for TTS. Estrogen had a small positive (non-significant) relationship with TTS and OAE shift. The effects of estrogen and progesterone on TTS and OAE shift were independent. Together estrogen and progesterone accounted for 66.5% of the

variance in TTS compared to 2% for estrogen alone and together accounted for 22.2% of the variance in OAE shift compared to 1.1% for estrogen alone. Hormones also impacted on TTS recovery with males having faster recovery than the early-follicular and oral contraception group despite having similar TTS.

The third aim was to determine whether the associations between auditory function measures (TEOAE amplitude, efferent suppression and 4 kHz threshold) and TTS and TEOAE shift differed in males and females. The results showed that the predictors of TTS and OAE shift did differ in males and females. In males there were no predictors of OAE shift. In females OAE shift was significantly predicted by OAE amplitude at 70 dB and efferent suppression between 60-65 dB. In males TTS was significantly predicted by 4 kHz thresholds and female TTS was predicted by 70 dB OAE amplitude. However, the direction of the association between the auditory measures, TTS and OAE shift was generally the same in males and females, with better auditory function as reflected by larger OAEs and better 4 kHz thresholds associated with more TTS and OAE shift, whereas stronger efferent suppression was generally associated with less TTS and OAE shift.

The fourth aim was to determine whether there was an association between estrogen and progesterone levels and auditory function measures in female participants. The results showed that progesterone was not associated with any of these baseline measures of auditory function. Estrogen is associated with all types of baseline measures of auditory function and particularly with OAE amplitude. High estrogen was generally associated with better auditory function as reflected by higher OAE amplitudes and better PTA thresholds, however in the case of efferent suppression high estrogen was associated with low suppression values.

The fifth aim was to determine whether estrogen and progesterone effected susceptibility to TTS and TEOAE shift through their effects on the auditory function measures or whether they acted independently to effect TTS and TEOAE shift. Linear regression models showed that the effects of progesterone on TTS and OAE shift were independent of the auditory measures. The effects of estrogen and auditory function on TTS were for the most part independent. Additionally, there

was no difference in the total variance in TTS explained by estrogen and progesterone together when adding any auditory measure to the model.

The addition of the auditory measures to the models had a greater impact on the amount of variance in OAE shift that was explained by estrogen than occurred in the TTS models. There was also an increase in the total variance in OAE shift explained when adding many of the auditory measures to the models, suggesting that the effects of these auditory measures and hormones are independent.

In summary, there was a mediating role of some aspects of auditory function on the effects of estrogen on TTS and to a greater degree on OAE shift. However, estrogen itself only had a very small impact on TTS and OAE shift so this suggests that for the most part the impact of auditory function and hormones on OAE shift are independent.

Aim 1: To determine whether males and females differed in TTS and TEOAE shift and recovery from TTS and TEOAE shift after exposure to a 3kHz pure-tone, 100 dB L_{Aeq}, 15 minute noise exposure.

This research showed no sex difference in the initial TTS or OAE shift following a 100 dB L_{Aeq}, 15 minute, 3 kHz pure-tone noise exposure. Initial TTS was around 12 dB (11.68 dB ± 6.01 for females and 12.47 dB ± 5.94 for males). Initial OAE shift which was first measured at 5 minutes post-exposure was around 2 dB (2.23 dB ± 1.69 in females and 1.66 dB ± 1.23 in males). Studies which have generated a similar amount of TTS to this research have also shown no sex differences in TTS (1, 27).

For example, there were no sex differences following a 4 hour, 100 dB L_{Aeq}, broadband music exposure which generated around 6.3 dB ± 3.9 of TTS at 15 minutes post-exposure (27). A comparison of data from this study at an equivalent post-exposure time (15 minutes 40 seconds) was performed to compare with the broadband noise study. There was a similar amount of threshold shift (female 5.33 dB ± 4.12, male 4.12 dB ± 3.69) and no significant sex difference: $t(33) = 0.910$, $p =$

0.370. There were also no sex differences in the TTS measured immediately (2 minutes) after a 1400-2800 Hz broadband noise exposure (3 minutes, 116 dB SPL) which generated around 14.2 dB of TTS (1), a similar amount to the initial TTS generated in this study.

Those studies in which a significant sex difference in TTS has been found in humans (1) or in animals (6, 115), have generated a higher level of TTS than occurred in this research. For example, females had a greater shift TTS (25.9 compared with 20.46) following a 2800 Hz pure tone exposure (3 minutes, 116 dB SPL) (1) which had an Leq of 94 dB whereas the exposure in this research had a Leq of 84.9 dB (208). This offers some support for the theory that sex differences in TTS are more pronounced at high sound levels.

There was a sex difference in TTS recovery: whereas female TTS was initially somewhat less than male TTS it was greater than male TTS from 9 minutes onwards and remained so for the length of the measured recovery time (46 minutes post-exposure). This indicates a slower recovery from TTS in females than in males. This contrasts with previous findings that either show no sex difference in TTS recovery (123) or that females had greater recovery from TTS (122). Both of these studies had higher initial TTS than occurred in this research (17-25 dB), however it is difficult to draw a conclusion with three different results from three different studies.

While there was a difference in recovery for TTS there was no sex difference in OAE shift recovery. To determine whether this difference is due to differences between males and females in OHC health, further analysis was performed of OAE shift recovery data. This was done as the TTS recovery groups included all the available participants as there were no missing data points due to un-recordable thresholds. In contrast, the OAE recovery groups only included those who had no missing data points due to low amplitude thresholds which did not meet SNR criteria. A larger number of male participants could not be included in the analysis due to missing data than female participants.

TTS recovery was compared in those 19 female participants and 14 male participants who could be included in the OAE shift recovery analysis, so that TTS and OAE shift recovery was compared in the same group of people. The same pattern was observed in this subset as occurred for the whole TTS recovery group: there was still an interaction of sex and post exposure recovery time indicating a slower recovery in female participants: $F(11, 341) = 4.472$, $p = 0.012$. The presence of a sex difference in the TTS recovery but not in OAE shift recovery suggests that the difference in TTS recovery is not driven by differences in the function of OHCs.

Aim 2: To determine whether there was an association between estrogen and progesterone levels and TTS and TEOAE shift in female participants and whether males, OC females and females in the early-follicular, late-follicular and luteal phases differed in TTS and TEOAE shift.

Assessment of the relationship between estrogen, progesterone, TTS and OAE shift showed that progesterone had a large, negative, significant association with TTS and to a lesser degree with OAE shift whereas the small positive relationship of estrogen with TTS and OAE shift was not significant. This association was shown by the presence of a difference in TTS between males and females in the luteal phase during which progesterone levels are highest, with luteal females having less TTS. The same pattern was observed in the OAE shift data but was not significant. There were also significant differences in TTS between females in the luteal phase and those in the late-follicular group who had the most TTS. During the late-follicular phase estrogen levels are at their highest and progesterone levels are low. Hormones also impact on TTS recovery with males having faster recovery than the early-follicular and OC group despite having similar initial TTS. There was less of a difference in OAE shift recovery amongst the hormone groups.

The finding that there is no difference in male and female TTS when females are treated as a whole group, but that there is a difference between males and females at particular phases of the menstrual cycle has been obtained previously. A previous study has found differences in TTS between males and females in the

early-follicular phase following a 110 dB SPL, 5 minute, white noise exposure (3), whereas another study using these exposure parameters in which females were not classified according to hormonal levels showed no apparent sex differences in susceptibility (135).

Previous inconsistencies in the presence or absence of a sex difference in susceptibility to NIHL could be related to this factor as well as or instead of differences in the response to different intensities of noise. For example, if a difference in susceptibility was small and variable: apparent during some stages of the menstrual cycle and not at others, such as occurred in this research, then a sex difference could easily have been missed, or its size underestimated (24). This emphasises the importance of quantifying female hormonal status when looking for sex differences in susceptibility to NIHL. If this had not been done it would have appeared that there was no sex difference in TTS amongst this group.

The direction of the relationship seen between TTS and menstrual cycle phase, whereby TTS is lowest during the luteal phase when progesterone is high, is a different relationship from that found previously (3, 4). These studies found that there was less TTS during the early-follicular phase (when both estrogen and progesterone are low) than in the luteal phase while other research has found no significant cyclical differences in TTS (122). The low TTS in those participants who were in the luteal phase was driven by the large, significant, negative correlation between progesterone and TTS and the lack of any significant relationship between estrogen and TTS. This was an unexpected result given the evidence showing plentiful ERs in the cochlea (7, 131, 132) which have been shown to have a role in protecting against noise induced threshold shifts (7) and other evidence which also shows that estrogen helps to protect the cochlea against noise induced damage (5, 6). In contrast, there are no published reports of progesterone receptors in the cochlea (133) and a preliminary investigation into the effects of progesterone pre-treatment on NIHL indicated that there was either no effect or a small worsening in noise induced threshold shift (6).

There are various possibilities as to why these results differ from these previous findings. These could include differences between the way that hormonal status was established in previous studies of hormonal status and TTS and how it was established in this study. It could also suggest that there are differences in the action of sex hormones during low levels of TTS inducing noise and high levels of PTS inducing noise.

Most previous animal research has suggested that estrogen has a role in modulating NIHL. Most has suggested that estrogen has a protective role (5, 6), although there is also some evidence that ovariectomy (which removes circulating estrogen as well as progesterone) has a protective effect against NIHL in the B6 mouse (8). Previous research in humans has indicated that females had less TTS in the early-follicular phase when estrogen levels are at their lowest (3, 4) whereas another found no cyclical difference (122). Much of this data does indicate that estrogen modulates NIHL, although the direction of effect does differ. This may be related to such things as species differences, with different experimental animals used in each study or to differences in experimental technique. For example, this type of research has examined the role of sex hormones and NIHL by comparing ovariectamised and intact animals (8), genetic modification of ERs (7), anti-estrogenic compounds (5) and estrogen pre-treatment (6). Additionally, the study which examined the effect of Tamoxifen on PTS used only male animals (5) and there may be differences in how estrogen interacts with noise in males due to the low and relatively stable levels of circulating estrogen and/or interactions with male dominant hormones such as testosterone.

Another explanation for differences in the effects of estrogen on NIHL may be related to the intensity of the noise exposure used to induce the NIHL. The animal studies which have found that estrogen was associated with a protective effect have used high levels of noise exposure which have caused a PTS (5, 6). The study which showed that ERβ knockout mice had greater threshold shifts (7) did use a TTS inducing noise like this study, although the TTS induced (25-27 dB) was higher than that found in this study or in the previous human studies which found that TTS was lowest when estrogen was lowest (3, 4).

Additionally, differences between this study and the previous human data may be due to difference in the manner in which hormonal status was established. Previous studies which have investigated cyclic variation in TTS in humans have not directly measured hormonal levels and have estimated hormonal status based on such methods as self-report of menstrual cycle stage (4) or by the measurement of basal body temperature and observation of cervical mucus quality (3). Classifying women into menstrual cycle stages based on indirect criteria such as the above provides only a rough estimate of hormonal status. Self reported menstrual cycle stage is a particularly unreliable method of estimating hormonal status and additionally menstrual cycle stages have been divided into phases differently which effects the days selected for testing and the associated predicted hormonal levels (24). The other methods of estimating menstrual cycle stage are still imprecise as there are very large inter-individual differences in the measured hormone levels of each menstrual cycle stage (24, 145). Direct measurement of hormone levels as performed in this research is recommended as the best way to establish any relationship between hormone levels and NIHL (24).

Progesterone effects

Discussion of the mechanism by which progesterone might impact on NIHL is very speculative as there has been no published research at all into the effects of progesterone and NIHL and little research into the impact of progesterone on normal auditory function. This lack of focus on the role of progesterone has also occurred in the central nervous system, although it has recently begun to receive more attention (209).

There are no published reports of progesterone receptors in the cochlea (133) which would suggest that progesterone is not acting directly through genomic or non-genomic means to modulate TTS. However, there are also no definitive reports of their absence and unpublished data from a thesis indicates that progesterone receptors may be present in the spiral ganglion cells of the human cochlea (134). This suggests that more studies with a range of different antibodies are required before determining the presence or absence of progesterone receptors in the cochlea (134).

The limited data on the role of progesterone on auditory function and NIHL is indicative of either no effect, or a worsening effect (6, 49, 137), although there is some data showing effects on cochlear blood flow (140, 141). The only data that directly considers the impact of progesterone on NIHL is a preliminary investigation which indicated that progesterone pre-treatment was associated with either no effect or a small worsening in noise induced threshold shift (6).

Importantly, progesterone has been shown to alter blood pressure and cochlear blood flow. It was shown to enhance the increase in blood pressure that was caused by a vasoconstrictive agent in the rat and to reduce the elevation in cochlear blood flow (141) and enhance the increase in blood flow that occurred after treatment with vasoconstrictive compounds in the guinea pig (140). This is one pathway through which progesterone could possibly act to effect NIHL.

There are some other theorised pathways by which progesterone could have effected TTS in a non-direct manner. For example, progesterone may have cross-reacted with other steroid receptors such as glucocorticoid receptors (210) which are present throughout the cochlea in varying amounts in humans and other mammals (211) and which seem to have a protective function against NIHL as when CBA mice were exposed to a TTS inducing noise a glucocorticoid agonist decreased TTS whereas a glucocorticoid antagonist worsened TTS (212). Additionally, progesterone or its metabolites such as allopregnanolene may have interacted with GABA-A receptors and acted as a GABA agonist (133). GABA is an important neurotransmitter of the auditory efferent system (77).

Alternatively, progesterone could have no effect on TTS but be associated with some other unmeasured factor which is associated with progesterone, for example, there are other hormones that alter over the menstrual cycle in addition to estrogen and progesterone such as ACTH and vasopressin (133).

Oral contraception and TTS

This study showed that OC females had TTS that was substantially larger than luteal TTS, however there were no significant differences between OC TTS and TTS

in the other groups including males. Previous research with OC females has shown that they had more TTS than males and MC females (122, 135). Other research has shown that OC TTS was greater than early-follicular TTS and similar during other phases (3).

The most common type of OC used in one of the studies was Ortho-Novum 1/50 (135) which contains 1mg of Norethindrone and 50 mcg of mestranol (3). The women in another study were using Ortho-Novum 7/7/7 (3) which contains 0.5 mg of norethindrone and 0.035 mg of ethinyl estradiol (213). Another study contained no information about the OC used (122).

The participants in this study used OC which differed in formulation from those above and also from each other. Details of contraceptive types are outlined in Appendix 4. 3/5 participants in this study who were OC users used OC with the same active substances, while the other two used different formulations. OC has been shown to alter function in ways that are dependant on the specific formulation of each type of OC (24). Because of this it is difficult to make meaningful comparisons between the TTS of OC users in this study and that obtained in previous studies.

Differences in the effect of hormones on OAE shift and TTS

There are numerous examples of a disassociation between the effect of sex and hormones on TTS and OAE shift: there is a significant sex difference in TTS recovery and not in OAE shift recovery, and there is a greater difference between male TTS recovery and the recovery of the female hormone groups than occurred with OAE shift. Additionally, the size of the association between progesterone and OAE shift is smaller ($r = -0.528$) and only marginally significant whereas than that between progesterone and TTS ($r = -0.789$) is larger and significant.

Various previous findings have also shown this dissociation between the effect of hormones on thresholds or measures of whole cochlear output such as the CAP and the effect on OAEs or measures of hair cell loss. For example, tamoxifen has

been shown to worsen CAP thresholds after noise exposure without significantly worsening DPOAEs (5). This is also shown after treatment with 17β estradiol where despite a protective effect of estrogen against PTS there was no difference in either OHC or IHC loss (6). There was also a dissociation between the effects of sex hormones on thresholds and hair cell loss following gonadectomy in the B6 mice but this study found a greater difference in hair cell loss than there was in threshold shifts (8).

The findings from this study are in line with those that suggest that hormones are more associated with sex differences in threshold shifts than OAE shifts, and that they may be exerting their effects on NIHL through such mechanisms as modulating the function of stria vascularis structures (137) or altering cochlear blood flow rather than through direct alteration of OHC function (6).

Aim 3: To determine whether the associations between auditory function measures (TEOAE amplitude, efferent suppression and 4 kHz threshold) and TTS and TEOAE shift differed in males and females.

There were differences between males and females in the auditory predictors of both TTS and OAE shift, however the direction of the association between the auditory measures, TTS and OAE shift was generally the same in males and females, with better auditory function as reflected by larger OAEs and better 4 kHz thresholds associated with more TTS and OAE shift, whereas stronger efferent suppression was generally associated with less TTS and OAE shift, this suggests that the same underlying processes are occurring in each sex. Females had a greater number of significant associations (which are listed in Table 4), apart from the association with 4 kHz threshold which was significant only in males, however there were greater numbers of female participants for each comparison.

In the case of 4 kHz 60 dB OAE amplitude males had a negative association whereas there was a positive association for females. However, as all the other correlations between OAE size, TTS and OAE shift were positive for both males and females then this result could be an error.

The direction of the associations between 4 kHz thresholds, TTS and OAE shift are generally in line with those found in previous research which has consistently found that better (lower) PTA thresholds are associated with more TTS (3, 4, 27, 28). This study also found that stronger efferent suppression was generally associated with less TTS and OAE shift (significant at 60-65 dB for female OAE shift). This is in line with the direction of the previous findings from animal research that have used PTS inducing noise exposures but in contrast to the human studies which have either found no associations between efferent suppression, TTS and OAE shift (166, 167) or a positive association (33).

The association between OAE size and TTS and OAE shift was in a different direction to that found between OAE size and PTS in previous studies where larger amplitude (better) OAEs were associated with less PTS (29, 30). This may be related to the fact that while these earlier studies looked at the relationship with PTS, this study examined the relationship with TTS. As outlined earlier, good auditory function (as reflected by low thresholds and high amplitude OAEs) may be associated with greater TTS as small amounts of TTS may be a reflection of the cochlea's ability to adapt when under stress to protect against further damage (9, 110). So, perhaps those with good auditory sensitivity have auditory systems that have a greater capacity to turn themselves down when stressed by small amounts of noise (reflected in higher amounts of TTS) and hence have less permanent hearing loss. This would suggest that when looking at the correlation between small amounts of TTS and OAE shift and measures of auditory function that better PTA and better OAE amplitudes should both be associated with more TTS and OAE shift. That was what was found in this thesis.

Aim 4: To determine whether there was an association between estrogen and progesterone levels and auditory function measures in female participants.

The results showed that progesterone alone is not associated with any baseline measure of auditory function. Estrogen by itself is associated with the full range of baseline measures of auditory function, and particularly with OAE amplitude where there was a significant or marginally significant association with estrogen at

each stimulus level. High estrogen was generally associated with better auditory function as reflected by higher OAE amplitudes and better PTA thresholds, however in the case of efferent suppression high estrogen was associated with low suppression values.

The lack of association between progesterone and these baseline measures of auditory function in this study is expected given that the previous study which has previously looked at this has also shown no association between progesterone and TEOAE amplitude (49) or efferent suppression magnitude (49). Other research has shown that there was a small and transient effect of progesterone on marginal cell function, but this occurred at very high levels of progesterone treatment (137).

This data showed that high estrogen was associated with low efferent suppression values at the 75 dB stimulus level. This is consistent with previous data which found a significant correlation between efferent strength and estrogen level during the follicular phase whereby efferent strength was lower when estrogen levels were high (49).

This data also showed that high estrogen was associated with higher OAE amplitudes. This is consistent with previous data which has also shown a small positive correlation between estrogen level and TEOAE amplitude during the follicular phase (49). This suggests that TEOAE amplitude should show cyclic variation across the menstrual cycle. Examination of Figure 46 which shows the TEOAE amplitudes for the different hormone groups shows that the late follicular group (highest estrogen levels) did have markedly larger OAEs than males and females in the early-follicular and luteal groups, however the pair-wise comparisons were not significant (perhaps due to the low N in the late-follicular group).

There have been contradictions in previous data with some suggesting that there are variations in OAE size throughout the menstrual cycle (50) as cited in (133), while others have found no fluctuation in OAE amplitude across the menstrual cycle, with both DPOAE and TEOAEs found to be stable in different investigations

(186, 187). Some have suggested that the sex difference in OAE size, which also occurred in this study with females having larger OAEs at lower stimulus levels, is related to organisational effects of hormones rather than activational effects (55, 174, 190). For example, pre-term infants have stronger TEOAEs from 35 weeks conceptional age (43), suggesting sex differences in OAE amplitude may be in place before the effects of circulating sex hormones come into play. However, the findings of this study confirm those (49, 50) that indicate that there are activational hormonal effects on TEOAE amplitude. This does not suggest that there are not also sex differences in OAEs due to organisational effects, as both types of hormonal effects can impact on the same phenomena (24).

In this study high estrogen was also associated with better (lower) 4 kHz PTA thresholds. There have been no investigations that associated PTA thresholds with estrogen levels but there is evidence that suggests that thresholds are better during times of the menstrual cycle when estrogen is higher than they are during the early-follicular period when estrogen levels are very low (3, 4). In contrast, some research has found that thresholds were better during the early- follicular phase (48). The data from this study is consistent with the former relationship.

Aim 5: To determine whether estrogen and progesterone effected susceptibility to TTS and TEOAE shift through their effects on the auditory function measures or whether they acted independently to effect TTS and TEOAE shift.

Linear regression models were developed to assess whether auditory function (as measured by efferent suppression amplitude, OAE amplitude and 4 kHz threshold) mediates the relationship between estrogen and progesterone on TTS and OAE shift. This showed that these auditory function measures do not mediate the effect of progesterone on either TTS or OAE shift. There was a mediating role of some aspects of auditory function on the effects of estrogen on TTS and to a greater degree on OAE shift. However, estrogen itself only has a very small impact on TTS and OAE shift so this suggests that for the most part the impact of auditory function and hormones on TTS and OAE shift are independent.

Previous authors have speculated that the relationship they found in which females had their worst thresholds during the early-follicular phase and also had less TTS during this phase meant that perhaps female sex hormones act to alter thresholds and then through this impacts on the amount of TTS (3). However, females during the early-follicular phase had similar thresholds to OC females and males but much less TTS whereas during the late-follicular and luteal phases female thresholds were better than OC and male thresholds but TTS was similar. They suggested that this indicates that female sex hormones also act independently of any threshold effect on TTS (3). The data from this thesis indicated that although the effects of estrogen on TTS and OAE shift are mediated somewhat by aspects of auditory function, the hormones generally have an independent effect on TTS and OAE shift.

Potential limitations

This study includes some limitations. These relate to the type of OAE chosen to monitor noise induced OAE changes, the absence of screening for normality of the menstrual cycle and low participant numbers.

Two types of evoked OAEs are generally used for the purposes of noise monitoring: DPOAEs and TEOAEs, and both have shown sensitivity to noise induced cochlear damage (214, 215). A choice was made to use TEOAEs as they have been shown to be more sensitive than DPOAEs in detecting sex differences in OAEs (178).

However, maximum TEOAE amplitude size is generally obtained at around 1-2 kHz and amplitudes taper off towards the high frequencies. TEOAEs are typically very small or absent in the high frequencies (from around 4.5 kHz). This is due to a combination of middle ear and response windowing effects as some high frequency energy is lost when windowing out the first 2.5 ms of the response (216). As this study involved a 3 kHz pure-tone designed to cause a TTS at 4 kHz this meant people could have low or absent OAEs at the frequency of interest which would mean that it would be difficult to detect noise-induced changes. 3/25

females and 4/21 male participants could not have OAE shift 1 measured due low amplitude or missing OAEs which made detection of an OAE shift impossible. Further participants had missing data for this reason when recovery from OAE shift was examined (6/25 females and 7/21 males).

Given that DPOAEs are measureable over a wider frequency range and can be measured in a specific frequency area at higher levels of stimulation than is possible with TEOAEs (217) it is possible that the use of DPOAEs may have resulted in a greater amount of usable OAE data.

Female participants were not screened for normality of menstrual cycle, menstrual cycle phase, contraceptive status or pregnancy. Consequently, females within the menstrually cycling groups could include those with abnormal menstrual cycles. In particular, those with low progesterone levels could include females who have failed to ovulate (anovulatory). The physiological environment may vary in these women compared to a normally cycling woman with equivalent estrogen and progesterone levels. Some things that have been shown to differ in anovulatory women such as an increased luteinising hormone to follicule stimulating hormone (FSH) ratio, decreased sex hormone-binding globulin and increased FSH (218). However, there is no data on how or even if these hormones would impact on the relationship of progesterone and/or estrogen on TTS.

Participant numbers were limited, particularly for comparisons between the menstrual cycle groups. Participant numbers were determined based on detecting a difference in TTS and OAE shift between males and females. This meant that the number of participants in the different menstrual cycle groups would be limited and uneven. Additionally, due to the large variability in hormone levels that can occur in the different menstrual cycle phases a conservative approach was taken when classifying participants into the different menstrual cycle groups and this meant that 4/26 participants in session 2 and 5/21 available participants in session 3 could not be unambiguously assigned into one group or the other. Furthermore, as two participants did not wish to have blood tests performed (both were non OC users and not pregnant) this also reduced participant numbers in the groups further. Participant numbers were particularly low in the late-follicular

group (N =2) and numbers in each group could be reduced further if there was missing OAE data in the various analyses.

Additionally, I was unable able to obtain the designated number of participants despite extensive advertising and an extension of the data collection time and an increase in the age limit to 35 to allow a greater number of people. This led to lower than ideal participant numbers even in the main male and female comparison groups.

The use of a TTS model in humans

This study investigated the impact of estrogen and progesterone on sex differences in susceptibility to NIHL using a TTS inducing noise exposure. This section will discuss issues related to the safety and suitability of exposing human participants to noise.

There are a number of threads of evidence which show that this noise exposure is well below the limit at which there is any reasonable potential for any on-going harm to an individuals auditory system. These include comparison with occupational safe noise exposure limits, previous research which has shown full recovery of function and comparison with animal based research which has shown synaptic changes and functional changes despite recovery of thresholds.

This study used a conservative noise exposure which was a 3 kHz continuous, pure-tone of 100 dB L_{Aeq} for 15 minutes. This exposure provided the equivalent energy to an eight-hour continuous A-weighted sound pressure level, $L_{Aeq,8h}$ of 85 dB which is the occupational safe noise exposure limit in New Zealand for an 8 hour work day as stated in Regulation 11 of the Health and Safety in Employment Regulations (1995) (118). This exposure was calculated to have an Leq of 84.9 dB (208) and provide a daily noise dose of 98% (119). This occupational safe noise exposure limit has been developed based upon a statistical analysis of risk to limit harm over the length of an individuals working life. It has been determined that after daily exposure to noise with equivalent energy to the noise exposure of this

research, that 95% of people would have little or no hearing loss with a threshold shift of less than 10 dB at the end of their working life whereas 5% would have a threshold shift of greater than 10 dB (120). Given that a working lifetime of exposure to noise of this level is associated with only a small risk of a threshold shift, there is no suggestion that a one-off exposure to that amount of noise is likely to cause any harm.

Additionally, previous research which has utilised noise exposures of a similar magnitude to this one have shown full recovery of auditory function. For example, full recovery of auditory function as measured by OAE levels occurred within half an hour of cessation of a noise exposure which was equivalent to an exposure level normalised to an 8 hour exposure of $L_{Ex,8h}$ of 86 dB L_{Aeq} (202). Furthermore, it has been found that a noise exposure causing a TTS at 2 minutes post exposure of 30 dB or less is fully reversible, this type of threshold shift might result from a 100 dB L_{Aeq} noise exposure of around 100 minutes duration (79), a considerably greater exposure than the exposure used in this research.

Although it seems clear from examination of the above factors that there is no reasonable potential for permanent shifts in thresholds or OAEs following a noise exposure of this level it is worth considering the recent evidence showing that there can be permanent harm to the auditory system following TTS inducing noise exposures that are not reflected in measures of thresholds or of OAEs (90, 91). This was initially found in the CBA/CaJ mice (90) and has been replicated in the guinea pig (91).

This research has shown that there can be supra-threshold deficits in functional measures of the auditory system with an associated loss of synaptic structures and subsequent loss of spiral ganglion cells in the presence of full recovery of thresholds and DPOAEs following noise exposure (90, 91). However, this phenomenon occurred after very large amounts of TTS which were near the threshold of reversibility. For example, there was around 40 dB of TTS at 24 hours post-exposure in the most effected part of CBA mouse cochlea (90). In those areas of the cochlea which were less effected by the noise exposure and which had a

smaller TTS (~ 20 dB at 24 hours) there were minimal synaptic changes, no supra-threshold reduction in ABR amplitude and no slow loss of spiral ganglion cells (90). This suggests that damage of this type most likely occurs subsequent to severe TTS.

Consideration of all this evidence shows that this study's noise exposure is well within the boundaries of safety. In this study recovery of thresholds was measured for 46 minutes post-exposure: at 46 minutes male TTS was 1.56 ± 2.668 dB and female TTS was 3.07 ± 3.100 dB, in comparison even when the TTS in the CBA mouse was still substantial at 24 hours post-exposure (20 dB) there was no sign of supra-threshold deficits in auditory function or loss of spiral ganglion cells (90). Additionally, the methodology of this study ensured that each participant received a 99 dB SPL noise exposure as measured at the ear drum. This resulted in audiometer presentation values ranging from 81 to 89 dBHL and ensured that no participant received an unexpectedly large noise exposure due to their ear canal characteristics which may have placed them at additional risk of harm.

Conclusions and implications

This first study into the effects of estrogen and progesterone levels on sex differences in human TTS and OAE shift found several novel and interesting findings. To summarise, it showed that there were no differences between males and females in initial TTS, OAE shift or OAE shift recovery, whereas females had slower recovery from TTS than males. The research also showed that progesterone levels mediate TTS, with high progesterone associated with low TTS. The same relationship occurs with OAE shift but is only marginally significant. This is a novel finding, as is the finding that estrogen had no significant relationship with TTS or OAE shift at this low level of noise exposure. Estrogen mediated different aspects of baseline cochlear function and hearing, whereas progesterone did not which confirms previous findings that have associated hormone levels with auditory function. There was no interaction between the effects of estrogen and progesterone on TTS. The suggestion of distinct roles for estrogen and progesterone within the cochlea is a also novel finding.

Additionally, there was a mediating role of some aspects of auditory function on the effects of estrogen on TTS and to a greater degree on OAE shift. However, estrogen itself only has a very small impact on TTS and OAE shift so this suggests that for the most part the impact of auditory function and hormones on TTS and OAE shift are independent.

These findings have implications for the understanding of the mechanisms on NIHL and well as for the management of NIHL through improved knowledge about the measurement of NIHL susceptibility.

NIHL mechanisms

One of the main findings of this study is that while there is variation in female TTS associated with progesterone levels, this variation is not associated with a sex difference (difference between males and the entire female group) in TTS.
One implication of this finding is that the sex differences in permanent NIHL are not generated by the activational effects of hormones at all. This might suggest that they are generated by some other mechanism such as activational hormonal effects on TTS duration or organisational hormonal effects which impact on body size and influence NIHL through proposed mechanisms such as differences in pinna size or projection, cochlear length or hair cell density.

The finding of no significant association between estrogen and TTS stands somewhat in contrast to previous research which has shown that estrogen does influence NIHL, however, these previous studies have used higher noise levels than this study. The contrast between the findings in this low-level noise exposure study and the previous studies using higher noise levels as well as this studies finding that estrogen influences baseline cochlear functioning could offer support for the theory that proposes different roles in the cochlea for ERα and ERβ receptor types. Based on findings showing changes in the expression of ERα, but not ERβ during the mouse menstrual cycle (191) and on results showing the importance of ERβ in the protective response to NIHL (7), it has been proposed that ERα and ERβ have different roles in the auditory system, with the role of ERα

to adjust auditory sensitivity based on the level of circulating hormones whereas ERβ is hypothesised to have a protective role which comes into effect when the auditory system is under stress such as responding to high levels of noise (191). These findings would suggest that the ERβ mediated protective mechanism would came into play at higher intensity levels than occurred in this study and if so this could If this effect might explain the difference in NIHL between males and females.

There is a sex difference in recovery from TTS which does seem to be hormonally based. This may indicate that sex differences in NIHL could be related to this rather than to the magnitude of the initial threshold shift. An association has recently been found in P2RX2 knockout mice in which they were found to have no TTS but a greatly increased risk of PTS. This suggests that TTS following low levels of elevated noise is a reflection of an adaptive response to noise whereby the cochlea reduces its own sensitivity, rather than a reflection of injury (110). By the same token, this extended period of TTS in females relative to males could suggest that they have a more robust and long-lasting adaptive response whereby sensitivity is reduced for a longer period, reducing the potential for further injury, which is associated with a decreased risk of PTS.

It has not been definitively determined at this time whether small amounts of TTS as obtained in this study are a reflection of an injury which recovers or whether it is simply a measure of an adaptive response as determined in recent research (110). Determination of this will need to await further knowledge about what factors are definitively associated with an injury process and what combination of noise frequency/intensity/duration and species factors leads to a helpful (or at least harmless) adaptive process occurring in the cochlea. In the case of the former this would mean that those females with high progesterone (luteal phase) and low TTS would be at reduced risk of any type of permanent injury relative to males whereas in the latter case luteal females would be at greater risk.

Measurement of susceptibility to NIHL in the workplace.

The management of NIHL risk in the workplace would benefit from an ability to quantify individual susceptibility to NIHL. A proposed method to do this is the use of a "hearing loss risk matrix". This can be used to target additional counselling and education and training at particularly susceptible individuals (51). For example, those who are at high risk for each factor in such a matrix could score 2 points, those for whom the factor is absent would score 0 with intermediate risk assigned a value of 1. Those individuals who score above a particular value that is set in the workplace or industry could receive counselling and explanation of those factors that place them at particular risk. They could receive more frequent audiological monitoring, re-evaluation of hearing protection type and training and be considered for other quieter jobs within the company (51).

The effective use of a matrix of this type requires significant research as to what susceptibility factors should be included, what weighting should be given to each factor and how the factors interact with each other (51). Consideration of the findings of this research which used a noise exposure with a similar energy level to what might be encountered in a work day can shed light on how the issue of sex should be handled within such a matrix.

Had the presence of estrogen and/or progesterone been associated with a lesser risk of harm for females in all menstrual phases this would have suggested that sex could have been a factor in the "hearing loss risk matrix", with pre-menopausal females assigned a 1 or 0 to indicate their lesser risk. However, the data shows that female TTS is significantly lower than male in the luteal phase, higher although not significantly so in the late-follicular phase and the same in the early-follicular phase. This current data would suggest that classifying susceptibility risk by sex would be inappropriate. Clearly the effect of hormonal factors is variable and will alter in a population according to phase of menstrual cycle. This is also shown by the absence of a difference between TTS in the male and entire female group. The hormonal effects may also alter according to the normality of the menstrual cycle, age (pre and post menopausal) and with other factors such as weight (218).

Other factors which may be also behind sex differences in susceptibility to noise such as organisational effects on body size, cochlear length and ear shape may be better to be classified in the "hearing loss risk matrix" according to measurement of that factor rather than by sex. Although sex differences are present in many of these factors in the population there is a large variation in size within the sexes (121).

Additionally, comparison of what were effective auditory predictors of susceptibility to TTS in males and females showed that while estrogen does mediate the size of many of these factors in females, that there is no great difference in what is an effective auditory predictor of NIHL in males and females. It was seen that the direction of the association between the auditory measures and TTS and OAE shift was generally the same in males and females, with better auditory function as reflected by larger OAEs and better 4 kHz thresholds associated with more TTS and OAE shift, whereas stronger efferent suppression was generally associated with less TTS and OAE shift. this suggests that the same underlying processes are occurring in each sex.

Suggestions for future research

This study has reinforced that the relationship between sex hormones and NIHL is clearly a complex one. Much remains to be known about how hormones interact with each other in the cochlea during different levels of noise exposure and how they interact with other processes to impact on NIHL.

While much research into the relationship between sex hormones and auditory function and NIHL has previously focused on estrogen, the results of this study indicate that there would be great value in further study of the role of progesterone in NIHL. It would be particularly interesting to know whether the association between progesterone and NIHL also occurred during higher levels of noise exposure and whether the theorised protective role of estrogen on NIHL following high levels of noise would occur. This research would likely need to

occur in an animal model to ensure that noise exposures could be loud enough to show a range of effects with changing noise levels.

Additionally, this study has shown an activational effect of sex hormones on TTS whereas the comparison between the whole female and male group showed no difference in TTS. However, evidence suggests that there is a difference in susceptibility to NIHL following long-term noise exposures (113, 114). This raises the possibility that male dominant sources of hormonal variability such as testosterone levels could explain differences in threshold shift between males and females. Testosterone levels have been shown to impact on OAE size, with higher levels of testosterone associated with lower OAEs in adult males (219), so there is a basis to query the role of testosterone in modulating threshold shifts.

While this study has shown an activational effect of sex hormones on TTS there was no difference between the sexes in TTS. This indicates that it would also be useful to clarify whether there are organisational hormonal effects on NIHL (24). The most appropriate group in humans to study the effects of prenatal androgen exposure is those females with congenital adrenal hyperplasia (CAH). This population have high exposure to androgens prenatally but receive treatment after birth meaning that androgen concentrations normalise (24). While this could theoretically be done in humans, the populations of people who could participate in a small country like New Zealand is very small. The best way to study these effects would be in an animal model. This way organisational and activational effects could be compared in the same species in conjunction with a range of noise exposure levels to compare the hormonal effects on TTS and PTS inducing noise exposures.

Conclusions

- While there is variation in female TTS which is associated with progesterone levels, this variation is not associated with a sex difference (difference between males and the entire female group) in TTS. This suggests that sex differences in permanent NIHL are not generated by the activational effects of hormones on initial TTS and may be generated by some other mechanism such as activational effects on TTS recovery or organisational hormonal effects which impact on body size and influence NIHL through proposed mechanisms such as differences in pinna size or projection, cochlear length or hair cell density.

- The contrast between the findings in this low-level noise exposure study and previous studies which have used higher noise levels, as well as this studies finding that estrogen influences baseline cochlear functioning could offer support for the theory that proposes different roles in the cochlea for ERα and ERβ receptor types. These findings would suggest that the ERβ mediated protective mechanism could came into play at higher intensity levels than occurred in this study and if so this could If this effect might explain the difference in NIHL between males and females.

- There is a sex difference in recovery from TTS which does seem to be hormonally based. This may indicate that sex differences in NIHL could perhaps be related to this rather than to the magnitude of the initial threshold shift. The extended period of TTS in females relative to males could suggest that they have a more robust and long-lasting adaptive response whereby sensitivity is reduced for a longer period, reducing the potential for further injury, which is associated with a decreased risk of PTS.

- This current data would suggest that classifying susceptibility risk by sex when trying to quantify individual susceptibility to NIHL would be inappropriate. The data shows that female TTS is significantly lower than male in the luteal phase, higher although not significantly so in the late-

follicular phase and the same in the early-follicular phase. Clearly the effect of hormonal factors is variable and will alter in a population according to phase of menstrual cycle. This is also shown by the absence of a difference between TTS in the male and entire female group.

- While estrogen does mediate the size of the auditory physiology measures that predict NIHL in females, there is no great difference in what is an effective auditory predictor of NIHL in males and females. It was seen that the direction of the association between the auditory measures and TTS and OAE shift was generally the same in males and females which suggests that the same underlying processes are occurring in each sex.

Glossary of terms

Activational effects: Hormonal effects that are due to the action of circulating levels of female sex hormones. The effects change depending on the level of the hormone (53).

Antioxidants: Molecules which inhibit ROS and convert them to less damaging molecules (97).

Auditory brainstem response (ABR): An auditory evoked potential which arises from the auditory nerve and the lower brainstem. It consists of a series of five to seven peaks and troughs (waves I – VII) that occur within 20 ms after stimulus onset (189).

A-weighting filter: An A-weighting filter is used as the ear is not equally sensitive to all frequencies of sound. The filter reduces the contribution to the overall sound level of the frequencies of sound to which the ear is less sensitive.

Compound action potential (CAP): An evoked potential which arises from the cochlear end of the auditory nerve as the nerve fibres leave the cochlea and enter the internal auditory canal. Equivalent to wave I of the ABR (189).

Coupler to dial difference (CDD): A transformation factor which accounts for the difference between the dial reading of an audiometer and the SPL in a coupler for the same stimulus (197).

Decibel (dB): Decibels are a relative measure of sound intensity which use a logarithmic scale (220).

dBA: Refers to sound intensity that has been measured using an A-weighting filter (221). Sound measured with an A-weighting filter is now often referred to with the notation dB $L_{Aeq.}$

dBHL (dB Hearing Level): The intensity of a sound measured in dB relative to audiometric zero: the SPL for a stimulus that a normal hearing person would be expected to hear 50% of the time (193).

dB SPL (dB Sound Pressure Level): The intensity of a sound measured in dB relative to a defined reference level. This reference level is 20 μPa (221).

dB SL (dB Sensation Level): The intensity of a sound measured in dB relative to the threshold level for that sound for a particular individual (221).

Distortion product otoacoustic emissions (DPOAEs): A type of evoked OAE measured in response to the simultaneous presentation of two different pure tones (f1 and f2) which stimulate two different places along the cochlea (66). This leads to the creation of a distortion product (DP) at another location along the basilar membrane that responds to a frequency that was not present in the two pure tones (67).

Efferent suppression: Efferent suppression is the altering (generally suppression) of cochlear transduction by either electrical or acoustical stimulation of the efferent neurons. The reduction on cochlear sensitivity can be measured clinically in a reduction of the amplitude of measures of cochlear function and output such as OAEs and CAP (74).

Endocochlear Potential (EP): The positive electrical potential of cochlear endolymph relative to the perilymph (+80 mV) (62).

Equal energy hypothesis: This hypothesis attempts to explain the relationship between the intensity and duration of a noise exposure and the subsequent noise induced cochlear injury and hearing loss. It states that equal amounts of noise exposure will produce equal amounts of damage: that is, a noise exposure of two hours will be equally as damaging as a noise exposure of 4 hours that is half its intensity and for noise of given intensity a 2 hour exposure will be twice as damaging as a 1 hour exposure (79).

Estrogen: One of the primary female sex hormones. The most common type of estrogen in the non-pregnant female is 17 β–estradiol (53).

Estrogen Receptor (ER): Receptors which are activated by the sex hormone estrogen. The two classes of ER are called ER α and ER β (53).

Follicular phase: The pre-ovulatory phase of the human menstrual cycle in which progesterone levels are low. Estrogen levels are initially low until a surge in the last-follicular phase in which estrogen levels reach their peak (53).

Free radicals: Molecules with an unpaired electron in their outer shell (97).

Gender: The classification of humans according to their "self-representation as male or female, or how that person is responded to by social institutions on the basis of the individual's gender presentation" (23), p 437.

Genomic effects: Genomic hormonal effects are initiated when the sex hormone binds to receptors in the cell cytoplasm. The hormone/receptor complex then migrates to the cell nucleus and alters gene expression (mRNA). This pathway has a timeframe of hours or even days (53).

Hughson-Westlake (modified) method: A method for obtaining audiometric thresholds. This involves lowering the sound in 10 dB intervals until there is no response and then increasing the sound in 5 dB intervals until a response is obtained. A threshold is obtained at the lowest level at which there is a response in at least 50% of a series of ascending trials with a minimum of two out of three responses at a particular level (193).

Luteal phase: The post-ovulatory phase of the human menstrual cycle. This phase is marked by a sharp increase in progesterone levels. Estrogen levels initially decrease and then rise. Both estrogen and progesterone levels decline at the end of the phase (53).

Non-genomic effects: Non-genomic hormonal effects are initiated when hormones bind to receptors in the cell membrane. These receptors activate 2nd messenger signalling pathways that have rapid effects. This pathway has a timeframe of seconds or minutes (53).

Organisational effects: Hormonal effects that are permanent and occur when hormones (testosterone) act on structures such as the genitals and brain in the foetus and neonate to cause a male phenotype resulting in permanently differentiated structures (53).

Otoacoustic emissions (OAEs): These are low-level sounds that originate in the healthy cochlea from the action of the OHCs either spontaneously (SOAEs) or in response to an evoking stimulus and which propagate out from the cochlea and through the middle ear and into the ear canal where they can be measured by sensitive microphones (65).

Pink noise: A noise whose SPL is constant across the octave bands (222).

Pure tone audiometry (PTA): PTA is a subjective behavioural test of hearing which involves the presentation of pure tones of different pitches and levels to determine the threshold of hearing (193) .

Progesterone: One of the primary female sex hormones (53).

Progesterone receptors (PR): Receptors which are activated by the sex hormone progesterone. The two classes of PR are termed PRA and PRB (53).

Reactive oxygen species (ROS): ROS are oxygen-based molecules. They include free radicals and molecules which are not free radicals but which easily generate free radicals such as hydrogen peroxide (97).

Real ear to coupler difference (RECD):The RECD is the difference between the SPL measured in an individual's ear canal and the SPL measured in a coupler for the same stimulus (197).

Sex: The classification of individuals "generally as male or female, according to the reproductive organs and functions that derive from the chromosomal complement" (23), p 437.

Spontaneous otoacoustic emissions (SOAEs): OAEs that originate spontaneously, without an evoking stimulus (65).

Susceptibility differences: Differences in NIHL despite exposure to an identical noise exposure (223).

Threshold of hearing: The lowest effective SPL of a stimulus that can evoke an auditory sensation (193).

Testosterone: The principle male sex hormone (53).

Transient evoked otoacoustic emissions (TEOAEs): A type of evoked OAE which is generally measured in response to a brief and abrupt click stimulus (65).

Appendix 1: Pre-selection questions

1.1 What is your date of birth?

1.2 What is your sex?

- *Male*
- *Female*

1.3 What ethnic group(s) do you identify with?

1.4 Are you currently or have you been exposed to noise at work so loud that it is difficult to hold a conversation?

- *No*
- *Yes, up to 20 hours a week*
- *Yes, over 20 hours a week*

1.5 How long have you worked in a noisy environment?

- *Under 3 months*
- *Over 3 months*

1.6 Have you been exposed to impulse noise (explosions, shooting etc.)?

- *Never*
- *Occasionally*
- *Quite often*
- *Often*

1.7 Have you ever played in any type of band or play a musical instrument?

- *Never*
- *Occasionally*
- *Quite often*
- *Often*

1.8 Have you ever used an iPod, other type of MP3 device, walkman or listen to amplified music?

- *Never*
- *Occasionally*
- *Quite often*
- *Often*

1.9 Have you ever been to night clubs, rock concerts, or other places with loud music?

- *Never*
- *Occasionally*
- *Quite often*
- *Often*

1.10 Have you ever used power tools, lawn mowers etc

- *Never*
- *Occasionally*
- *Quite often*
- *Often*

1.11 Have you ever had recurrent ear infections or other problems with your ears?

- *Yes*
- *No*
- *Don't know*

1.12 Have you ever been hospitalized for a head injury?

- *Yes*
- *No*
- *Don't know*

1.13 How well do you think you can hear?

- *Good*
- *Fair*
- *Poor*

1.14 Do you ever have tinnitus (sounds in your ears/head)?

- *Never*
- *Occasionally*
- *Often*
- *Always*

1.15 Have you ever taken medicines that affect your hearing?

- *Yes*
- *No*
- *Don't know*

1.16 Do you have a family history of hearing loss?

- *Yes*
- *No*
- *Don't know*

Appendix 2: Coupler to dial difference

	500 Hz	1 kHz	2 kHz	3 kHz	4 kHz
CDD 1	6	1.75	7	5.75	0
CDD 2	6	1	6.5	5.25	0.5

Coupler to Dial Difference values. The CDD was measured at the beginning of data collection (CDD 1) and was re-measured after the audiometer received its scheduled calibration (CDD 2). Each value is the average of two measurements.

Appendix 3: Estrogen and progesterone levels and hormone group classification

	Session Two			Session Three		
	E	P	Phase	E	P	Phase
F1	218	0.49		300	0.7	
F2	16798	79.5	PR	193	8.3	L
F3	90	0.49	F(e)	184	0.5	
F4	188	1				NT
F5	1580	1.1	F(l)	494	38.2	L
F6	502	0.49		480	0.9	
F7	107	0.6	F(e)	77	0.7	F(e)
F8	189	5.2	L			NT
F10	49	0.49	OC	49	0.49	OC
F11	49	0.49	OC	49	0.49	OC
F12	896	0.7	F(l)	236	0.5	
F13	160	9.8	L	194	29.8	L
F15	280	12.5	L			NT
F16	83	0.8	F(e)	83	1.3	F(e)
F17	10233	119.4	PR	16704	127.2	PR
F18	90	0.49	F(e)	373	37.4	L
F19	49	0.49	OC	49	0.6	OC
F20	495	69.2	L	270	0.5	
F21	213	12.5	L	105	0.8	F(e)
F22	97	0.5	F(e)	250	34.3	L
F23	49	0.49	OC	49	0.49	OC
F24	173	13.5	L			NT
F25	49	0.49	OC	49	0.49	OC
F26	190	4.3		123	0.6	F(e)

Estrogen (E) and Progesterone (P) levels and hormone group classification. Estrogen values are reported in pmol/l and progesterone values in nmol/l. PR = pregnant, F(e) = early-follicular, F(l) = late-follicular, L = luteal, OC = oral contraceptive users, NT = not tested. F9 and F14 are not included as they did not receive blood tests. The phase tab has been left empty if the participant could not be unambiguously allocated into a menstrual phase group.

Appendix 4: Participant's contraception types

- Two participants were using Ginet-84 which contains 2 mg of cyproterone acetate 2mg and 0.035mg of ethinyl estradiol per tablet (224).
- Another participant used Estelle-35ED which contains the same active substances as above (225).
- Another participant used Norimin which contains 500 mcg of norethisterone and 35 mcg of ethinyl estradiol (226)
- Another participant used Levlen ED which contains 0.15 mg of levonorgestrel and 0.03mg of ethinyl estradiol (227).

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