Trauma and Residual Hearing Loss
After Cochlear Implantation Surgery

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Thesis Summary

For the last 30 years, cochlear implantation (CI) surgery has been successfully used for the treatment of severe to profound deafness. With improvements in cochlear implant technology, the surgical criteria have expanded to include patients with residual low frequency hearing. These patients can benefit from simultaneous use of the implant and a hearing aid in the same ear, termed electro-acoustic stimulation (EAS). EAS has been shown to be particularly beneficial for perceptual tasks relying on pitch resolution, such as music appreciation and speech perception in background noise (Gantz et al., 2005, Santa Maria et al., 2013). Unfortunately, residual hearing loss, termed post-implantation hearing loss, occurs in half of all patients, which degrades many of the benefits of EAS (Carlson et al., 2011).

Post-implantation hearing loss may arise from direct surgical trauma and indirect damage to the cochlea (Eshraghi and Van de Water, 2006). The causes of direct trauma include surgical access to the cochlea and electrode insertion trauma. This results in several types of tissue injury, which may cause further hearing loss through a biological response, also termed indirect damage. However, the precise contributions of direct and indirect damage to post-implantation hearing loss remain indeterminate, in part, because of the lack of a standardised animal implantation model. Animal experimentations have been partly hampered by the difficult access to the mammalian inner ear and the absence of a consistent implantation technique (Rowe et al., 2016, Smeds et al., 2015, James et al., 2008) that has led to marked differences in the level of trauma and hearing loss (O'Leary et al., 2013, Farhadi et al., 2013, Honeder et al., 2015).

The principal aim of this thesis was to examine the different types of cochlear trauma, how they relate to hearing loss and how hearing loss can be prevented. The issues that have been addressed here include cochlear anatomy and imaging techniques, pathology, pharmacology, electrophysiology and force recordings. This thesis begins by establishing a reproducible animal model of cochlear implantation surgery, followed by systematically examination the relevant contributors to surgical trauma and post-implantation hearing loss. From these findings, pharmacological therapy targeting the indirect damage and recording techniques to reduce post-implantation hearing loss have been investigated.
An anatomical study of the guinea pig cochlea was initially performed using novel imaging and processing techniques. This study quantitatively described the soft and bony tissue relationships in the complex hook region of the unoperated guinea pig cochlea. A reproducible technique for implanting in an animal was established in this study for the remaining *in vivo* studies, including the use of a cochleostomy in an anteroinferior plane (highest predictability and smallest risk of trauma) and closer to the round window (lowest force profile).

The first *in vivo* experiment of this thesis investigated the relative influence of implant insertion depth on trauma and hearing loss. This study also explored the use of glucocorticosteroids (‘steroids’) to preserve hearing and target the biological response to the implant. Key findings were that implants deeply inserted or in the presence of an osseous spiral lamina fracture caused the greatest low-frequency hearing loss. Steroids reduced the biological response in the most apical regions but had no effect on hearing thresholds. A second *in vivo* experiment was performed to further investigate the efficacy of steroids and to explore if trauma and hearing loss could be predicted at the time of surgery through force or electrocochleography recordings. Preserving the compound action potential of the electrocochleography recording was found to be predictive of an atraumatic insertion and reduced post-implantation hearing loss. Conversely, force was correlated with trauma but not hearing loss. Pre-operative steroids were effective in reversing the loss of hearing amplitudes evoked by lower frequency tones during implantation with hard electrodes.

The results presented in this thesis will help inform the cochlear implant community of potential techniques to improve hearing preservation surgery. Specifically, these results provide a validated experimental model of hearing preservation surgery. These results also suggest a role for steroids in reducing tissue response and synaptopathy, and for electrocochleography as an intraoperative recording paradigm to reduce trauma and improve post-implantation hearing loss.
Declaration

This is to certify that:

(i) This thesis comprises only my original work towards my PhD except where indicated in the Preface

(ii) Due acknowledgement has been made in the text to all other material used

(iii) This thesis is less than 100,000 words in length, exclusive of tables, bibliographies and appendices

Jonathon Lo

August 2018
Preface

The following unchanged multi-author papers were included in this thesis:

   - In addition to fulfilling the criteria for authorship, I would like to acknowledge the contributions of: PS, LC with Amira and thin sheet laser imaging microscopy; SW with MatLab and mathematical modelling; SC with animal handling; SOL and HE with conceptualisation, analysis and editing

   - In addition to authorship, I would like to acknowledge the contributions of: PS, LC with Amira and thin sheet laser imaging microscopy; AH and SC with animal handling; SOL and HE with conceptualisation, analysis and editing. I would also like to thank Helen Feng for manufacturing the electrode arrays; Rachel Sore of the Statistical Consulting Centre (University of Melbourne) for guidance on statistical analysis; and, Sjaak Klis (University Hospital Utrecht, Netherlands) for providing the gold ball recording electrodes.

   - In addition to authorship, I would like to acknowledge the contributions of: CB, AC with MatLab and intraoperative electrocochleography recordings; AH with animal handling; CN with electrode design and force recordings; SOL and HE with conceptualisation, analysis and editing. I would also like to thank the contributions of: Helen Feng for manufacturing the electrode arrays; Sjaak Klis (University Hospital Utrecht, Netherlands) for providing the gold ball recording electrodes; Hanif Miah (Cochlear Limited) for assistance with Instron recordings; Dimitra Stathopoulos for
the epoxy cochlear model.

No other work towards this thesis has been submitted for other qualifications. No work towards this thesis was carried out prior to enrolment in the degree. No third party editorial assistance was provided in preparation of this thesis.
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List of Abbreviations

ANOVA - analysis of variance
ABR - auditory brainstem response
ANN – auditory nerve neurophonic
CI – cochlear implantation
CM – cochlear microphonic
CO - cochleostomy
CAP – compound action potential
CT – computed tomography
dB- decibel
EAS - electro-acoustic stimulation
ECochG - electrocochleography
OSL – osseous spiral lamina
RWM – round window membrane
Hz – Hertz
kHz – kilo Hertz
kg – kilogram
LASER - light amplification by stimulated emission of radiation
L – litres
µm – micrometre
mg - milligram
ml – millilitre
mm – millimetre
mN – millinewton
ms - millisecond
mV – millivolt
mW - milliwatt
min – minute
nm - nanometre
N – Newton
s- second
SEM – standard error of the mean
SPL – sound pressure level
SP – summating potential
V – Volt
Chapter 1: Introduction to Trauma in Cochlear Implantation Surgery
Chapter 1: Introduction to Trauma in Cochlear Implantation Surgery

For the last 40 years, cochlear implantation (CI) surgery has enhanced hearing for patients with profound hearing loss. To date, more than 300,000 hearing impaired individuals have become recipients of a cochlear implant (Roland and Tobey, 2013). Over the years, considerable improvements in cochlear implant technology has led to the expansion of the surgical criteria to include patients with residual low frequency hearing. These patients benefit from simultaneous use of the implant and a hearing aid in the same ear, variously termed electro-acoustic stimulation (EAS), hybrid CI or partial deafness CI. EAS with preservation of residual hearing has been shown to be particularly effective for difficult perceptual tasks relying on temporal aspects of sound perception, such as listening in noise or to music (Gantz et al., 2005, Santa Maria et al., 2013). Despite best efforts, the potential for EAS are greatly diminished in approximately half of patients because of loss of the residual hearing, termed post-implantation hearing loss (Carlson et al., 2011).

Post-implantation hearing loss may occur from direct surgical damage and indirect damage to the cochlea (Eshraghi and Van de Water, 2006). However, the precise contributions of direct and indirect damage to post-implantation hearing loss remain indeterminate, in part, because of the lack of a standardised animal implantation model. For example, variations in experimental technique (Rowe et al., 2016, Smeds et al., 2015, James et al., 2008) have resulted in marked differences in the level of trauma, hearing loss (O'Leary et al., 2013, Farhadi et al., 2013, Honeder et al., 2015) and hearing protection afforded by pharmacological therapies, like glucocorticosteroids (Lee et al., 2013, Connolly et al., 2011, Kuthubutheen et al., 2015).

The purpose of this thesis was to reconcile some of these inconsistencies by exploring the nature of surgical trauma with regards to hearing preservation cochlear implantation surgery. The following introduction begins by exploring the anatomy and physiology of hearing, along with objective measures of hearing. It then outlines the status of cochlear implantation research, including the expanded criteria of CI surgery. Next, the patterns and causes of post-implantation are explored, including the surgical approach and implant design. The types of direct surgical trauma are then detailed, including macroscopic and molecular types, followed
by the histopathological response to trauma. The remainder of this literature review explores current approaches to reducing surgical trauma and post-implantation hearing loss, including pharmacological protection and monitoring techniques. This chapter concludes with an overview and the aims of this thesis.
1.1 **Anatomy of hearing**

Physical sound can be considered as a wave motion of pressure differences that propagates away from its source. Sound can also be considered as an excitation of the auditory system that results in its perception. As such, the anatomy of hearing is intimately related to the physical properties of the wave motion, along with psychoacoustics and excitation of the auditory system. The loudness of a given sound is in relation to the amplitude of the pressure difference, while pitch is determined by the frequency of the vibration. The human auditory system is capable of hearing sounds with a loudness of up to 120 decibels and across a frequency range of 20 to 20 000 Hz.

The three principle components of the auditory system are the external ear, middle ear and inner ear (or cochlea). Perception of sound begins with transmission through the external and middle ear, followed by processing in the cochlea, the sensory end organ of hearing. The auditory system is designed to funnel, filter, amplify and convert sound wave pressure in to electrical impulses that are conveyed along the auditory pathway to the brainstem for further processing. In general, conductive hearing loss occurs when sound is unable to be transmitted because of a deficit in the external or middle ear. Conversely, a sensorineural hearing loss occurs from a deficit of the cochlea or further along the central nervous system.

**1.1.1 External ear**

The external ear is composed of an auricle (or pinna) leading to the external auditory canal (meatus externa). The auricle operates as a sound-gathering device and is involved in directional hearing (for example, from behind or in front of a person) and spatial clues, provided by both ears working together. The auricle focuses sound into the external auditory canal and provides amplification in a frequency-specific manner. Similarly, the external auditory canal, with radius of 3.5 mm and length of 25 mm, increases the loudness of sounds traversing it. The external auditory canal is open at one end (near the auricle) and closed at its inner end by the tympanic membrane (or eardrum). As such, the canal has acoustic similarities to an organ pipe, with the resonance effect increasing sound pressure at the tympanic membrane at certain frequencies. The maximum increase in sound pressure occurs with the mid-frequencies occupied by speech because of their relative wavelength, which
Unfortunately makes the ear more susceptible to hearing loss at these frequencies.

1.1.2 Middle ear

The middle ear is an air-filled cavity containing a chain of 3 delicate bones, termed ossicles, which are named malleus, incus and stapes. The function of the middle ear is to transform, with maximum efficiency, the vibratory energy of the tympanic membrane to the fluid of the cochlea. This is performed by mechanical linkage, such that vibrations from sound are transferred from the tympanic membrane to the adherent handle of the malleus, then on to the incus and subsequently the footplate of the stapes that adjoins the oval (or vestibular) window of the cochlea. Since the oval window is flexible and fluid filled on its inner ear surface, the tympanic membrane and ossicles act as a transducer of airborne sound to liquid pressure differences at the oval window.

The middle ear is often referred to as a mechanical-impedance-matching device because of its transformation of sound from the low impedance medium of air in to the high impedance of cochlear fluids. The impedance ratio between air and water is about 4 000:1. Hence, a direct air-fluid interface would result in a substantial reduction in the intensity of sound reaching the inner ear (an approximately 40 dB loss). The most important feature of the mechanical-impedance matching device is the amplification that occurs when sound energy is applied to a relatively large surface of the tympani membrane (about 80 mm$^2$) and applied to a much smaller area of the oval window (3 mm$^2$). Other amplification features include the lever arrangement of the malleus and incus (ratio of 1.4:1) along with vibrations of the tympanic membrane that act against the compliance of the trapped air in the middle ear.

1.1.3 Inner ear

The cochlea (Latin for ‘snail’) is the sound-analysing organ embedded in the temporal bone of the skull. The cochlea shares the same fluids with the adjacent vestibular system (the balancing organ), but their functions are independent. The cochlea is a spiral-shaped and fluid-filled tube with a length of 34 mm in humans and about $2 \frac{3}{4}$ turns (Rask-Andersen et al., 2012). It is divided lengthwise by a structure termed the organ of Corti, which performs the principal function of the cochlea: transduction of mechanical sound waves in to electrical signals. In turn, these electrical signals travel as action potentials along the neural auditory
pathway for processing.

Initially, vibrations are transmitted from stapes at the oval window on to a fluid filled compartment on the opposite side of the oval window, termed scala vestibuli (see Figure 1.1). Scala vestibuli is in continuity with another compartment, termed scala tympani, by the helicotrema located at the most apical end of the cochlea. At the basal end of scala tympani is a membranous structure, the round window membrane (RWM), located in proximity to the stapes. Both scalae are filled with perilymph, a sodium rich fluid that communicates with cerebrospinal fluid via the cochlear aqueduct. Due to the incompressibility of fluids, an inward movement of the oval window (following vibration of the tympanic membrane and ossicles) results in a displacement (outward movement) of the flexible RWM, and this is known as the round-window reflex.

A third compartment, scala media (or cochlear duct), is apportioned from the other scalae by two membranes: the basilar membrane (between scala tympani and media) and Reissner’s membrane (between scala media and vestibuli; also termed vestibular membrane). Scala media contains a potassium rich fluid called endolymph that differs in composition to perilymph and allows the formation of an electrochemical potential (see 1.1.4 Sterocilia). The apical end of scala media also ends blindly near the helicotrema while the basal end of scala media terminates blindly in the vestibular cecum, except for a small membranous tube that is termed ductus reuniens. The ductus reuniens communicates with the saccule of the vestibular system.

Vibrations from the wave in perilymph evokes a similar displacement, in the form of a traveling wave from the base to apical end of the cochlea, in both endolymph and the basilar membrane. The basilar membrane is attached from the spiral ligament of the lateral wall of scala tympani to the osseous spiral lamina (OSL) throughout its length (Figure 1.1). The basilar membrane is composed of radially directed filaments and a basement membrane. These components progressively change in density and width in such a way that the basilar membrane is narrow and stiff at the base while being wide and flexible at the apex. This arrangement causes a traveling wave to have a maximal displacement along the basilar membrane that varies depending upon the frequency of the exciting sound initiating the wave. As such, high-frequency sounds result in a peak amplitude near the cochlear base at the oval window; low-frequency sounds produce maximal amplitude at the apical end of the basilar
membrane; mid frequencies lie somewhere in between. The frequency causing maximal displacement of the basilar membrane at a specific cochlear place is called the characteristic frequency and this frequency-place organisation (the basis for the ‘place theory’ of hearing) is referred to as ‘cochleotopic’ (*topos* is Latin for place). The traveling wave will reach its maximal amplitude at the cochlear frequency then rapidly dissipate, like an ocean swell breaking on the shore. In the setting of complex signals, such as music or speech, myriads of momentary peaks are produced along the basilar membrane with each frequency removed from the composite traveling wave after its peak has been attained. The linear distance of the basilar membrane has roughly a log relationship with characteristic frequency such that each octave of frequency corresponds to approximately the same distance along the basilar membrane.

**Figure 1.1** Schematic diagram of the cochlea and organ of Corti that demonstrates the fluid compartments and their partitioning via basilar and Reissner’s membranes. Reprinted from: *Hearing and Deafness*, by Davis H and Silverman SR, 3rd ed, 1970, New York: Holt, Rinehart and Winston.

The travelling waves on the basilar membrane stimulate sensory hair cells and these convey signals to the brain in the form of neuron discharges. Hair cells are functionally arranged within the organ of Corti and supported atop the acellular basilar membrane (Figure 1.1). The organ of Corti is arranged as a single row of inner hair cells nearest the modiolus and three to five adjacent rows of outer hair cells. Hair cells owe their name to the tiny protruding...
hairs, termed stereocilia, that are numerosely arranged in a bundle (see 1.1.4 Stereocilia).

The inner hair cells act as microphones or transducers that convert vibrations of the basilar membrane and cochlear fluid to electrical signals via stereocilia. Inner hair cells synapse with the terminal dendrites of the spiral ganglion cells, which coalesce to form the auditory nerve, also termed the cochlear nerve. The auditory nerve exits the cochlea at its central axis, the modiolus, and traverses the internal acoustic meatus on its way to the brainstem. The outer hair cells have unique motility properties owing to the motor protein prestin and they function to provide amplification or attenuation to more sharply tune the output of inner hair cells. Their motility is an active process requiring metabolic energy, therefore, outer hair cells may be particularly vulnerable to hypoxia and ischaemia. Anatomical relationships of the cochlear hook region pertaining to surgery are detailed in later sections (incl. 1.4.4 Cochleostomy vs round window).

1.1.4 Stereocilia

As sound drives movement of the cochlear fluids and basilar membrane, the stereocilia of hair cells are deflected according to the vibrations arounds them. This occurs because stereocilia are covered by the tectorial membrane, a gelatinous structure that is attached to a thickening of the OSL, termed the spiral limbus. Stereocilia are ordered in graded height along the apical pole of the hair cell, with the tallest stereocilia contacting the tectorial membrane. Displacement of the basilar membrane from a travelling wave will cause a movement relative to the tectorial membrane because of their differing points of attachment. This divergence leads to a shearing motion on the stereocilia and this movement is converted into electrical signals through mechanically gated non-selective cation channels. Extracellular filaments (‘tip links’) connect shorter to longer stereocilia and coordinate the opening of cation channels when the bundle is deflected towards the large stereocilia and closure of channels when deflected away. Current due to this ionic flow generates a voltage and when above a certain threshold, will depolarize hair cells with deflections in the direction of the larger stereocilia. Conversely, deflections in the opposite direction of the larger stereocilia hyperpolarise the hair cell. This process arises from the presence of an electrochemical gradient, termed the endocochlear potential, between endolymph (at the apex of the hair cell) and perilymph (at its base). The endocochlear potential is approximately 80 mV and exists because of the high levels of potassium within the endolymph compared to the perilymph.
Generation of the endocochlear potential is not well understood. However, scala media and more specifically stria vascularis, located on its lateral wall and containing a rich plexus of intraepithelial capillaries, is believed to play an important role through the electrogenic extrusion of potassium into endolymph (Patuzzi, 2011).

Depolarisation of the inner hair cells leads to release of the neurotransmitter glutamate which travels across the synaptic cleft to the dendrites of bipolar cochlear nerve cells, located at the basal pole of the hair cell. The dendrites of these cells pass medially in canals in the osseous spiral lamina. The bodies of the cochlear nerve cell, located in Rosenthal’s canal, spiral alongside the modiolus and are thus named spiral ganglion cells. A single hair cell synapses with 10-20 spiral ganglion cells while a single spiral ganglion cell receives input from more than one inner hair cell. Frequency-place organisation occurs in spiral ganglion cells in a similar fashion to inner hair cells. In response to transmitter release from the inner hair cells, the peripheral axons of spiral ganglion cells initiate action potentials that propagate along through the soma and central axons to the cochlear nucleus, the first of a series of auditory brainstem nuclei. Besides providing frequency cues, information of loudness is also conveyed through an increased rate of, and number of nerves that are firing according to the sound input. The brainstem nuclei provide processing of the sound input, including frequency composition, temporal features and directionality of sound. Signals are finally projected to the auditory cortex, still with frequency-place organisation, for higher order processing of sound.

The role of the membrane potential generated by outer hair cells is still being investigated but is unlikely to be involved in encoding sound stimuli. The membrane voltage modulation is correlated with the expansion and contraction the outer hair cells due to the presence of prestin, a motor protein of the cell membrane that changes shape with voltage, much like a piezoelectric actuator. The mechanical response of outer hair cells to sound serves to amplify, and provide a local feedback control to, basilar membrane displacement. This process results in a 20 to 80 dB amplification depending on the frequency of sound (amplification is greatest at high frequencies) and is one of the main determinants of the sharp tuning and high mechanical sensitivity of mammalian hearing. A notable derivative of the outer hair cell response is a retrograde basilar membrane movement that results in vibration of the tympanic membrane, leading to the production of sounds that are termed otoacoustic emissions.
1.1.5 Measuring the functionality of the auditory system

Auditory function can be assessed by subjective methods that rely on behavioural responses or by objective measures that make use of physiological responses. Commonly used tests include speech and pure tone audiometry. Speech audiometry is indicative of the severity of functional impairment, while a pure tone audiogram evaluates perceptive threshold levels to a pure tone stimulus across a range of frequencies. Another used auditory stimulus is a click, which is a multi-frequency stimulus that elicits a broad response from the cochlea.

Electrocochleography

Objective tests of auditory function have been used where behavioural measures are difficult or not feasible, such as the screening of hearing in newborns and for auditory assessments in animal research. One of the earliest developed objective tests is electrocochleography (ECochG), which is recognised as an excellent measure of cochlear function. For example, the ECochG waveform is able to capture the various neural responses of the cochlea, including from receptors to nerve cells. The ECochG potential (see Figure 1.2, Figure 1.3) consists of four main responses: cochlear microphonic (CM), summating potential (SP), compound action potential (CAP) and auditory nerve neurophonic (ANN). ECochG potentials have been recorded from electrodes positioned on the skin, or via trans-tympanic (Yoshie et al., 1967, Schoonhoven et al., 1996) or extra-tympanic methods (Ferraro, 2010). More recently, recordings have been performed directly from the cochlea using either an electrode placed at the RWM (Mandala et al., 2012, Radeloff et al., 2012, Dalbert et al., 2015), an electrode placed inside of it (Calloway et al., 2014), or via an implanted electrode (Campbell et al., 2015, Campbell et al., 2016). Alternating stimuli will usually be presented for ECochG recordings and the resultant 2 ‘phases’ (or polarities), termed condensation and rarefaction, are added to derive a CAP and ANN, while subtracted for the CM (Bester et al., 2017).

The CM (Figure 1.2) is an alternating current potential that reflects the instantaneous displacement of the basilar membrane (Ferraro and Durrant, 2006) and follows the waveform of the acoustic stimulus. The CM is generated chiefly by the outer hair cells (Dallos, 1986) and its spatial localisation corresponds to the traveling waveform. A secondary component of the alternating current is the ANN, which is a phase-locked neural fibre response, similar to when presented with a sustained stimulus (Chatrian et al., 1984). The ANN is usually derived

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by summing the alternating phase responses and isolating the 2\textsuperscript{nd} harmonic, which is a two times multiple of the stimulus frequency (Weinberger et al., 1970).

![Figure 1.2](image)

**Figure 1.2** Single phase cochlear microphonic recording evoked by tone bursts. Reprinted (Ferraro, 2010, p150).

The SP (Figure 1.3) is stimulus related, like the CM, but is a direct current shift that is contributed more by inner hair cells (Durrant et al., 1998). The polarity of this baseline shift varies with stimulus frequency, intensity and location of the recording electrode (Ferraro and Durrant, 2006).

The CAP (Figure 1.3) represents the summed response of synchronised auditory nerve fibre activity. The CAP is characterised by a series of brief, predominantly negative deflections that are representative of the distribution of neural firing (Ferraro and Durrant, 2006). With suprathreshold stimuli, the first and largest of these peaks is referred to as N\textsubscript{1} and occurs with synchronous neural firing at about 1ms after CM onset. A second deflection, N\textsubscript{2}, occurs with a second synchronous firing of nerve fibres about 2ms after the CM onset (Ferraro and Durrant, 2006). The magnitude of the CAP response reflects the number of nerve fibres firing, while the latency of the response represents the time between stimulus onset and the peak of N\textsubscript{1}. Where present, CAP thresholds have been shown to strongly correlate with behavioural thresholds arising from pure tone audiometry (Schoonhoven et al., 1996, Wong et al., 1997). This correlation is strengthened when ECochG recordings are made closer to the cochlea (Schoonhoven et al., 1996).
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Figure 1.3  Showing ECochG evoked by click stimuli. The baseline of the recording is shown (Base). This is followed by the summatting potential (SP), a direct current shift. Two negative deflections of the CAP are shown (N₁, N₂). Reprinted (Ferraro and Durrant, 2006, p46).

In addition to identifying deficits of the auditory system, ECochG is used clinically to identify hydrops in Meniere’s patients (Ferraro and Durrant, 2006) and more recently, in the study of auditory neuropathy spectrum disorder (Rance and Starr, 2015). While Meniere’s and other disease pathologies have relied upon the SP (and rarely the ANN), the application of ECochG for hearing loss has primarily focused on CAP and CM responses (Ferraro, 2010).

Other objective tests of auditory function

Another widely used objective test of auditory function is the auditory brainstem response (ABR), which is an evoked potential generated by brainstem auditory nuclei in response to acoustic stimulation. The ABR is recorded using a differential amplification with reference electrodes placed around the ear and with an active electrode positioned at the vertex. It is a far field evoked potential, as opposed to the near field responses recorded with ECochG, and this may lead to the detection of activity evoked via the contralateral ear unless these are masked, contributing to false threshold recordings (Irving et al., 2014). However, previous studies have shown similar reliability for assessing auditory function with either ABR or ECochG recordings (Arslan et al., 1983, Quddusi and Blakley, 2009).

Distortion product of otoacoustic emission (DPOAE) is another objective test of hearing that occurs as a by-product of the electromechanical transduction of the outer hair cells. In response to sound, outer hair cells are believed to propagate a reverse movement of the
basilar membrane towards the cochlear base and through the ossicles, resulting in vibration of the tympani membrane (Ohyama et al., 1991). This vibration results in faint but audible sound that is termed an otoacoustic emission. DPOAEs are typically used only to classify hearing status as within normal limits or impaired (at given test frequencies) and do not predict hearing threshold levels, especially when losses are greater than 50 dB (Gorga et al., 1997).

**Hearing loss grades**
The severity of hearing loss is ranked according to hearing thresholds, measured in decibels of hearing loss (or dB HL), across several frequencies. For many years, hearing loss has been ranked differently according to the organisation and so research groups will often use varying systems (Clark, 1981). Hearing loss classifications have typically consisted of slight or mild, moderate, severe and profound grades of hearing loss. The classification used by the World Health Organization is shown in **Table 1.1**. Hearing loss may be present in one ear (termed unilateral) or in both ears (termed bilateral) and at different levels of severity. ‘Disabling hearing loss’ is defined as hearing loss greater than 40 dB in the better ear in adults and greater than 30 dB in the better ear in children (WHO, 2018). Notably, pure tone audiometry (in a quiet environment) is not a reliable indicator of hearing disability, particularly since communication in background noise is the primary complaint of implant recipients (Tavora-Vieira et al., 2016) (see **1.3.1 Expanding the cochlear implantation criteria**).

<table>
<thead>
<tr>
<th>Hearing Loss Grade</th>
<th>Range (dB HL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight/mild</td>
<td>26-40</td>
</tr>
<tr>
<td>Moderate</td>
<td>41-60</td>
</tr>
<tr>
<td>Severe</td>
<td>61-80</td>
</tr>
<tr>
<td>Profound</td>
<td>&gt;81</td>
</tr>
</tbody>
</table>

**Table 1.1** Grades of hearing impairment according to decibels of hearing loss (dB HL). Reprinted (WHO, 2018).

**1.2 The cochlear implant**
Chapter 1: Introduction to cochlear implantation and trauma

The cochlear implant was the first neural prosthesis to successfully enhance a sensory organ and is the optimal technique for restoring hearing to profoundly deaf people (Clark, 2008). The cochlear implant stimulates the auditory nerve electrically, patterned to encode the acoustic stimulus, thus bypassing damaged portions of the cochlea. The implant device consists of two main components: an external component consisting of a microphone, sound processor and transmitter system; and, internal components consisting of a surgically fixed receiver/stimulator and an electrode that is implanted within the cochlea.

The notion of electrical methods for inducing hearing began in 1790 when the Italian physicist Alexander Volta stimulated his own auditory system by passing current across his head (Clark, 2008). The sound was instantaneous but brief and likened to ‘boiling, thick soup’ (Mangus et al., 2012). In 1957, the first electrical stimulation of the auditory nerve was performed on a deaf subject by French doctors André Djourno and Charles Eyries (Djourno and Eyries, 1957). The patient could detect large changes in frequencies and distinguish certain words (e.g. ‘papa’, ‘maman’, ‘allô’) but had otherwise limited speech perception. The success of Djourno and Eyries generated global interest in electrical stimulation to restore hearing. This included a group consisting of Dr William F. House (an otologist), Dr James Doyle (a neurosurgeon) and Jack Urban (an engineer), who commenced implantation on volunteers in the U.S.A. Single channel implant devices were initially used and ranged from electrodes placed directly on the acoustic nerve to gold coated electrodes placed within the cochlea. Most patients could detect differences between many environmental sounds and experienced significantly improved lip-reading and communication (Roland and Tobey, 2013). This group’s work was met with scepticism amongst the scientific community until independent research in 1975 showed considerable speech perception benefits among 13 of their recipients (Roland and Tobey, 2013).

During the 1970’s, Ingeborg Hochmair and Erwin Hochmair (with other Austrian colleagues) made crucial advancements in the safety and tolerance of cochlear implants (Roland and Tobey, 2013). This group were also able to successfully stimulate the cochlea from an electrode placed within it. Similar developments were made by the University of Melbourne’s Department of Otolaryngology, led by Professor Graeme Clark. Both teams developed an array of multiple electrodes (termed a multichannel cochlear implant) imbedded in a silicone carrier that could be inserted in to the cochlea (Clark, 2008). The multichannel implant utilised the cochleotopic organisation of the cochlea to stimulate cells in frequency-
specific regions of the cochlea. At the same time, developments were made in the speech processor that encodes the acoustic information into electric impulses, which enabled dramatic improvements in speech recognition. Blake Wilson, an electrical engineer, was central in the development of a speech coding strategy that is still fundamentally used 25 years later (Roland and Tobey, 2013).

Modern cochlear implant systems consist of a microphone that converts sound into an electrical signal (Figure 1.4). This signal is selectively filtered by a speech processor that is then converted by a receiver-stimulator into electrical impulses. These electrical impulses are fed to the electrode array, which is embedded in the cochlea to stimulate the spiral ganglion neurons. The microphone and processor are housed together in a ‘behind-the-ear’ unit, while the receiver-stimulator package is surgically placed beneath the skin and against the skull. Transcutaneous radio signals are transmitted from an external coil to an internal receiving coil on the receiver-stimulator. The ground (reference) electrode is placed remotely from the cochlea, usually under the temporalis muscle.

![Diagram of an in-situ cochlear implant](image.png)

**Figure 1.4** Diagram of an *in-situ* cochlear implant, showing the external sound processor (plus microphone and transmitter), along with the internally placed receiver and electrode array that is inserted into the cochlea. Reprinted (MedlinePlus, 2017).

### 1.3 Electroacoustic stimulation and post-implantation hearing loss
1.3.1 Expanding the cochlear implantation criteria

Cochlear implantation (CI) surgery was originally performed to aid the lip reading and general sound awareness of patients with severe to profound hearing loss who had little or no speech recognition abilities. Over time, improvements in the design of implants resulted in dramatic enhancement of postoperative speech recognition (Holden et al., 2013) and as consequence, the indications for CI surgery were expanded to include patients with progressively more speech recognition abilities (Arnoldner and Lin, 2013). Surgical candidacy was also gradually broadened to include patients with a single sided deafness (Arnoldner and Lin, 2013), a shortened period of prior hearing loss (Blamey et al., 2013) and more recently, significant residual low frequency hearing. Residual low frequency hearing is a relatively common pattern among adults with a sensorineural loss. Sometimes the low-frequency preservation is excellent, with these patients exhibit a ‘ski-slope’ audiogram that drops precipitously at high frequencies. For these patients, minimal improvement is seen with acoustic amplification using a hearing aid, because key speech components, including consonants, cannot be delivered to these patients (Hogan and Turner, 1998).

Initially, the criteria for operating on patients with residual low frequency hearing were close to conventional CI surgery. This included patients with a hearing loss of 65 dB or more (in hearing thresholds) for frequencies below 1kHz and little or no hearing (i.e. ≥ 70dB HL) above 1 kHz (von Ilberg et al., 1999). However, these recipients were found to have much higher levels of speech recognition than from a well-fitted hearing aid (Turner, 2006) and this led to an even broader inclusion of patients with moderate to no hearing loss (i.e. <60 dB HL) below 1kHz (Gifford et al., 2013, von Ilberg et al., 2011) (see **Figure 1.5**). It was found that many of these recipients benefited from electrical stimulation of middle and high frequency sounds along with the complementation of low frequency amplification through the hearing aid and this combination strategy has since been termed hybrid stimulation, partial deafness cochlear implantation, or more commonly, electric acoustic stimulation (EAS).
In most audiological centres, the preselection criteria for EAS have evolved from primarily audiogram findings to a greater emphasis placed upon speech recognition scores under everyday, real-life conditions (von Ilberg et al., 2011). Today, several pre-operative hearing tests are used, including monosyllable words at normal conversational speech in quiet, and speech and sentence recognition in noise (Tavora-Vieira et al., 2016). Some authors have also proposed universal speech perception scores for guiding EAS criteria (Tremblay et al., 2008, Skarzynski et al., 2015, von Ilberg et al., 2011, Leigh et al., 2016). As an example, Von Ilberg et al. (2011) advocate for best aided scores in a monosyllable word recognition test to be ≤50% (at 65dB SPL). However, any formal selection process should be mindful of features specific to the audiological test, the implantation device and patient considerations, such as hearing-aid use and the status of contralateral hearing (Govaerts, 2016).

1.3.2 Performance benefits of electroacoustic stimulation

Electroacoustic stimulation was originally envisioned to improve all aspects of implant performance. However, this has not been proven to be true for standard word and sentence recognition in quiet (Friedland and Runge-Samuelson, 2009). Instead, EAS has been
demonstrated to be most beneficial over electrical stimulation alone for perceptual tasks relying on pitch resolution, such as listening in noise or to music (Gantz et al., 2005, Turner et al., 2004, Lenarz et al., 2013, Santa Maria et al., 2013, Brockmeier et al., 2010, Gifford et al., 2013). These benefits include improved localization with multiple noise sources (Dunn et al., 2010) and enhanced melody recognition (Dorman and Gifford, 2010, Gfeller et al., 2007, Gfeller et al., 2012). The mechanisms behind the benefits of EAS are largely unknown but it is believed that the interaction of electric and acoustic cues via place coding along the auditory pathway plays a significant role (Irving et al., 2014).

The improved implant performance has been shown to have broader benefits for the recipient, including higher income, better employment prospects (Roland, 2005, Monteiro et al., 2012), and an improved quality of life (Gstoettner and Arnoldner, 2008). Unfortunately, more than half of recipients experience some loss of residual hearing loss after surgery and this degrades many of the perceptual and performance benefits (Choudhury et al., 2014). The full preservation of residual hearing, termed hearing preservation, has therefore emerged as a contemporary goal of CI surgery and has been the focus of recent research.

1.3.3 Residual hearing preservation – defining ‘success’

Until recently, no universal classification system for hearing preservation existed. Previously, several centres used their own classification system by testing at various frequencies and often relying upon features specific to the type of audiometer (such as the cut-off limit) and the user’s initial hearing (Skarzynski et al., 2013). Ultimately, this has rendered the EAS literature difficult to interpret and with few meaningful comparisons across centres (Santa Maria et al., 2014). A classification system was recently devised by a collaborative group of experts (Skarzynski et al., 2013) (see Figure 1.6) that uses standardised octave intervals of the audiometer (125-8000 Hz). The goal of this classification system was to make it applicable for all subjects with residual hearing. The resultant equation is expressed as a percentage and classified as complete, partial, minimal or no hearing preservation (Table 1.2). A variation of this proposed classification includes further defining complete and partial preservation (Santa Maria et al., 2014), although this is also yet to be universally adopted.
Figure 1.6 Determining hearing preservation on a numerical scale, as proposed by Causon et al. (2015). S is the preservation on a numerical scale (%); $PTA_{post}$ is the postoperative hearing threshold, $PTA_{pre}$ is the preoperative hearing threshold and $PTA_{max}$ is the maximum measurable threshold. Note, PTA = audiogram pure tone average of 250, 500, 1000, 2000 Hz (Skarzynski et al., 2013).

As an example of this hearing preservation equation, patient $AB$ undergoes cochlear implantation with a pure-tone audiogram average of 55 dB pre-operatively ($PTA_{pre} = 55$) and 30 dB at post-operative time interval ($PTA_{post} = 30$). If the maximum measurable threshold is 120dB, then $AB$ has had $62\% (=1 - \frac{25}{65})$ of their residual hearing preserved at that particular time interval. Based on Table 1.2, this is classified as a partial hearing preservation.

<table>
<thead>
<tr>
<th>Percent of residual hearing preserved</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;75%</td>
<td>Complete HP</td>
</tr>
<tr>
<td>&gt;25-75%</td>
<td>Partial HP</td>
</tr>
<tr>
<td>0-25%</td>
<td>Minimal HP</td>
</tr>
<tr>
<td>No measurable hearing</td>
<td>Loss of hearing/no hearing</td>
</tr>
</tbody>
</table>

Table 1.2 Scale for a hearing preservation (HP) classification proposed by Skarzynski et al. (2013) and based on the above equation.

Two recent reviews on EAS have shown the rate of hearing preservation to be highly variable both within and between studies. For example, a meta-analysis of 110 EAS subjects found a median hearing preservation of 62% (Causon et al., 2015). Subjects showed extreme differences in hearing results, even within the same study, ranging from none (0%) to complete hearing preservation (100%) (Causon et al., 2015). A separate review that defined ‘complete hearing preservation’ to be within 10 dB of the patient’s preoperative hearing,
found complete hearing preservation among larger studies to range from none to half of their respective patient cohorts (Santa Maria et al., 2014). Notably, 8 out of 20 studies had no subjects with complete hearing preservation (i.e. all recipients with more than 10 dB of hearing loss) (Santa Maria et al., 2014). However, speech perception in background noise was still significantly improved among 2 of these studies (Santa Maria et al., 2013, James et al., 2006), which suggests that a 10dB threshold loss may not be a clinically relevant definition.

1.4 Post-implantation hearing loss

1.4.1 Patterns of post-implantation hearing loss

Successful hearing preservation is reliant upon the prevention of residual low frequency hearing loss, also termed post-implantation hearing loss. Post-implantation hearing loss is recognised as consisting of two patterns, immediate and delayed. Immediate, or ‘early’, post-implantation hearing loss is detected at the first follow-up audiological appointment, occurring within 1-month of surgery (Gantz et al., 2009). Delayed, or ‘long-term’, hearing loss arises in patients that have initially preserved their residual hearing but experience losses in the weeks-months after surgery (Dedhia et al., 2016). Delayed hearing loss has been further categorised as ‘fluctuating’ (Gstoettner et al., 2004, Woodson et al., 2010), ‘stable’ (also termed progressive or gradual) or ‘sudden loss’ (Gifford et al., 2013, Dedhia et al., 2016, Gantz et al., 2009). The detection and categorisation of these delayed forms hearing loss is largely dependent upon the frequency of post-operative audiological testing, which varies greatly between centres and has resulted in their underappreciation (Quesnel et al., 2015). Furthermore, the overall rate of post-implantation hearing loss (and successful hearing preservation) is likely to be underestimated since many clinical studies have a short observation period that fails to capture all forms of hearing loss (Santa Maria et al., 2014).

1.4.2 Causes of post-implantation hearing loss

Immediate post-implantation hearing loss is believed to arise from trauma-associated stimuli and the activation of cell-death pathways (Dinh et al., 2008). Trauma may occur at any stage of the procedure, including in the early stages from acoustic trauma and vibrations arising from drilling on or near the cochlea (Zou et al., 2005). However, most trauma is believed to
occur from the insertion of the electrode (see 1.4.5 Electrode insertion trauma). Trauma-associated stimuli may cause direct injury to the basilar membrane and sensory cells. The lymphatic channels and blood vessels may also be injured (Wright and Roland, 2013), and this can cause direct hearing loss, like with ischaemia, or indirectly through changes to the endocochlear potential (Reiss et al., 2015).

Trauma-associated stimuli may also instigate an inflammatory reaction and oxidative stress, which can lead to further hair cell death (Bas et al., 2012) or to a loss in function, such as pre- and post-terminal synaptic losses (Reiss et al., 2015) (see 1.6 Histopathological response to trauma and the electrode). As an example, pro-inflammatory stimuli like tumour necrosis factor α, can result in hair cell death by inducing apoptosis, necrosis or necrosis-like cell death pathways (Eshraghi and Van de Water, 2006). A more recently proposed cause of post-implantation hearing loss is the development of endolymphatic hydrops (Smeds et al., 2015), defined as an increased volume of scala media that is suspected to arise after injury to the ductus reuniens (Richard et al., 2012, Quesnel et al., 2015, Handzel et al., 2006) or from inflammation.

Compared to immediate post-implantation hearing loss, the aetiology and mechanism of delayed losses are less appreciated. Delayed hearing loss has been observed to occur at a rate far greater than can be expected through either age-related loss (termed presbycusis) or the natural progression of disease (Santa Maria et al., 2013, Yao et al., 2006, Gifford et al., 2013). Delayed effects are theorised to result from an intracochlear tissue response, in particular, the development of fibrotic changes around the electrode, new bone growth and the foreign body reaction (O'Leary et al., 2013, Nadol et al., 2008). The biological activity of the tissue response may cause a delayed degeneration of hair cells, of spiral ganglion neurons or of the synapse (Eshraghi et al., 2007b), thus resulting in a long-term hearing loss. Another proposed mechanism of delayed hearing loss is from the tissue response dampening cochlear mechanics, such as with fixation of the basilar membrane or RWM (O'Leary et al., 2013, Choi and Oghalai, 2005). Excitotoxicity from chronic stimulation, in a manner similar to noise-induced hearing loss, has also been proposed as a potential cause of delayed hearing loss (Kopelowich et al., 2015, Tanaka et al., 2014).

1.4.3 Surgical factors for post-implantation hearing loss
Techniques to reduce surgical trauma and the resultant inflammatory responses of CI surgery were first proposed by Lehnhardt in 1993 (Lehnhardt, 1993). Termed ‘soft surgery’, these interventions have changed little from their original description (Friedland and Runge-Samuelson, 2009) and have been widely adopted by implant surgeons. Most techniques have some correlation with post-operative hearing outcomes, although few have been validated in a randomised controlled trial. A case series of revision CI surgery found that bone dust, produced during the creation of the mastoidectomy or cochleostomy, was found to promote an extensive inflammatory response and could also result in a conductive hearing loss (McElveen et al., 1995). Today, the introduction of bone dust is strictly avoided. Similarly, the entry of blood is largely prevented. An animal study by Radeloff et al. (2007) found that even small amounts of intracochlear blood caused a permanent low frequency hearing loss, perhaps by providing a scaffolding for the ingress of a tissue response (Kel et al., 2013). Direct suctioning of perilymph from the cochleostomy or RWM has been found to result in immediate hearing loss (Mandala et al., 2012). Although, this has not been conclusively correlated with long-term hearing loss, it is typically avoided (Garcia-Ibanez et al., 2009). Surgeon will often use a sealant, such as harvested muscle graft from temporalis or connective tissue (Brown et al., 2010), in order to prevent perilymph loss, however, this has also been proposed as a cause of delayed hearing loss (Rowe et al., 2016).

Vibrations and noise levels during mastoidectomy have been extensively examined \textit{in vivo} and \textit{ex vivo} (Eshraghi et al., 2005). Drill-induced noise exposure can cause temporary losses in hearing (Migirov and Wolf, 2009, Kylen and Arlinger, 1976) but can be reduced by drilling intermittently, at low speeds (Hilmi et al., 2012) or with alternative instrumentation. For example, drilling of the last shell of bone of the cochleostomy can cause a noise exposure of more than 130 dB SPL (Pau et al., 2007), therefore, opening of the last shell is often performed with a non-drill instrument such as a micro-hook.

Inserting against resistance is avoided because of the results of temporal bones that have shown direct trauma arising at the electrode tip, such as elevation or penetration of the basilar membrane or trauma to the lateral cochlear wall, or buckling at the base of the electrode (Roland and Wright, 2006, Mukherjee et al., 2012). Topical use of the lubricant Healon®, comprised of sodium hyaluronate, has been consistently shown to reduce resistance during electrode insertions (Kontorinis et al., 2011b) and this has led to widespread use in hearing preservation CI surgery (Friedland and Runge-Samuelson, 2009). A slower insertion speed

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has been shown to reduce insertional forces (Kontorinis et al., 2011a). However, a slow insertion has not been directly demonstrated to improve hearing preservation nor the likelihood of a complete insertion. Although a small clinical study using historical controls did suggest that a vastly slower speed, up to 11 times slower than conventional insertions, may improve the probably of improving hearing preservation (Rajan et al., 2013).

1.4.4 Cochleostomy vs round window

Scala tympani has been consistently demonstrated to be the optimal electrode location for patients with normal anatomy (Finley et al., 2008, Skinner et al., 2007, Aschendorff et al., 2007, Holden et al., 2013). Scala tympani can be accessed by its natural boundary, the RWM, or by drilling an overlying segment of bone (the promontory) in a procedure termed a cochleostomy. The round window is found at the beginning of the hook region, a fish hook-like structure approximately 2.5mm in length (Skinner et al., 2007) that sharply curves in three directions before commencing in to the spiral shape of the cochlea (Stidham and Roberson, 1999, Atturo et al., 2014). Electrode insertions, placed via a RWM approach and successfully navigating the hook region, have been shown to be largely dependent on the electrode type. For example, straight electrodes can be inserted via the round window with minimal risk of trauma to the hook region (Briggs et al., 2006). On the other hand, contour electrodes placed via the round window (see 1.4.6 Electrode array designs) have been found in some studies to have a high risk of surgical trauma (Souter et al., 2011).

The cochleostomy technique has been found to successfully accommodate a variety of electrode designs (Briggs et al., 2005) and is the more extensively studied surgical approach. A cochleostomy is performed on the promontory, distant to the RWM, and theoretically provides the advantage of an insertion vector that is better aligned to the longitudinal axis of scala tympani (Breinbauer and Praetorius, 2015, Adunka and Buchman, 2007). A notable disadvantage of the cochleostomy approach is that it requires accurate placement to achieve a direct entry into scala tympani (Briggs et al., 2005, Li et al., 2007a) and to prevent immediate intracochlear damage (Breinbauer and Praetorius, 2015, Atturo et al., 2014). Structures at risk from poor placement include the osseous spiral lamina, basilar membrane and spiral ligament that have been shown to course anterior and superior to the round window (Li et al., 2007b, Stidham and Roberson, 1999). Hence, utilisation of a superior or overly anterior cochleostomy have been shown to result in highly traumatic insertions, including direct scala
media or vestibuli insertions (Lee et al., 2011, Briggs et al., 2001, Adunka et al., 2007). Cochleostomy placement in an excessively inferior plane may theoretically risk damage to the cochlear aqueduct and inferior cochlear vein (Li et al., 2007b, Guo et al., 2016), although this has not been reported in post-mortem temporal bones. Anatomical modelling (Li et al., 2007b, Guo et al., 2016) and implantation studies using ex vivo temporal bones (Briggs et al., 2005, Adunka et al., 2007) have shown the anteroinferior plane to be the safest plane for cochleostomy placement.

While cochleostomy placement has been extensively evaluated in terms of its ideal plane, the optimal distance along the hook region has not yet been assessed experimentally. Anecdotally, several studies have estimated cochleostomy placement to be up to 1mm from the edge of the round window niche (Berrettini et al., 2008, Lee et al., 2010, Kiefer et al., 2004). However, most authors are less specific and identify placement as being somewhat close to the round window (Brown et al., 2010, Carlson et al., 2011, Gifford et al., 2013, Gantz et al., 2005, Garcia-Ibanez et al., 2009). Depending on the surgical view, a subset of surgeons will place the cochleostomy adjacent to the round window such that drilling of the edge of the round window is required to expose scala tympani. This procedure has been variously termed ‘peri-round window’ (Zhou et al., 2014), ‘extended round window’ (Angeli and Goncalves, 2016), ‘marginal’ or ‘round-window related’ cochleostomy (Adunka et al., 2014). Hearing comparisons using this approach compared to a separate cochleostomy have not yet been performed, however, a single temporal bone study showed similar rates of surgical trauma (Zhou et al., 2014). The effect of cochleostomy size on hearing loss has not been determined but most surgeons opt for a drill size that is much larger than the electrode (typically 1.2mm diameter compared to 0.4mm), to improve the ease of insertion (Iseli et al., 2014) and to limit intracochlear fluid pressure changes that may occur with electrode insertion (Todt et al., 2014, Mittmann et al., 2017).

Whether cochleostomy has better hearing preservation than the round window approach has been the subject of ongoing debate. Inserting via the RWM negates some of the vibration and noise exposure associated with drilling of the cochleostomy (see 1.4.3 Surgical factors for post-implantation hearing loss). However, many surgeons that opt for a RWM approach will still drill a small segment of bone that may overhang the round window niche and/or part of the round window membrane for adequate visualisation (Iseli et al., 2014). Two meta-analyses showed conflicting benefit with each approach (Santa Maria et al., 2014, Causon et
al., 2015), although these studies differed in their definition of hearing preservation and criteria for inclusion. Overall, these meta-analyses, coupled with the results of single centre studies (Doshi et al., 2015, Adunka et al., 2014), demonstrate that hearing preservation is achievable through either approach and without clear evidence of benefit for any single technique.

1.4.5 Electrode insertion trauma

Archival temporal bones of recipients that were implanted with early designed implants have provided useful insight into the various types of gross structural disruption. Modern imaging techniques have provided further insights of the correlation between structural integrity and hearing (Finley et al., 2008), while experimental surgeries have led to the increasing recognition of trauma at the microscopic or molecular level as an important contributor of post-implantation hearing loss (Reiss et al., 2015, Tanaka et al., 2014, Kopelovich et al., 2015).

Gross morphological trauma: types and grading

Commonly reported trauma among the temporal bone collections of deceased recipients include fracture of the OSL, disruption to the basilar membrane (Nadol et al., 2001, Lee et al., 2011, Richard et al., 2012) and translocation into scala vestibuli (SV) (Nadol et al., 2001, Lee et al., 2011). Localised damage to the spiral ligament (Eshraghi et al., 2003, Linthicum and Galey, 1983), secondary tears of Reissner’s membrane and dissection of the lateral wall of scala tympani (Nadol et al., 2001, Cervera-Paz and Linthicum, 2005, Lee et al., 2011) have also been reported. Eshraghi et al. (2003) thus devised a scale of cochlear implantation trauma (Table 1.3) based upon early post-mortem findings and from insertional studies in to cadaveric temporal bones. The devised scale has enabled consistent reporting between studies, however, the scale is arbitrary and without any correlation to post-implantation hearing loss.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histopathologic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No trauma</td>
</tr>
<tr>
<td>1</td>
<td>Elevation of basilar membrane</td>
</tr>
<tr>
<td>2</td>
<td>Rupture of basilar membrane or spiral ligament</td>
</tr>
<tr>
<td>3</td>
<td>Translocation into scala vestibuli</td>
</tr>
</tbody>
</table>

Chapter 1: Introduction to cochlear implantation and trauma
Fracture of osseous spiral lamina or modiolar wall

Table 1.3 A trauma scale that compares grade of trauma (0-4) with the histopathological change after cochlear implantation. Adapted (Eshraghi et al., 2003, p150).

Although the precise contributions of gross morphological trauma on post-implantation hearing loss remain uncertain, most are likely to be enormously detrimental. Basilar membrane damage may vary from minor contact interfering with cochlear mechanics and/or the spiral vessels on its underside (Roland and Wright, 2006, Choi and Oghalai, 2005, Kiefer et al., 2006), through to severe disruption with mixing of endolymph and perilymph. Intermixing of cochlear fluids disrupts the endocochlear potential and has been shown to result in auditory hair cell loss in animals (Shaddock et al., 1985, Gulya et al., 1990). OSL (Grade 4) has been shown in experimental animal models to be associated with neuronal loss (Clark, 1977, Shepherd et al., 1983), presumably from severing of the dendrites of spiral ganglion cells (Roland and Wright, 2006). A fracture may also cause the formation of new bone, which may interfere with cochlear mechanics (O'Leary et al., 2013). Translocation of the electrode in to scala vestibuli (Grade 3) requires profound disruption of the OSL and/or basilar membrane (see Figure 1.7). Scala vestibuli translocation is one of the few types of gross electrode insertion trauma that can be imaged in vivo and has been found to adversely affect the functional hearing of implant recipients (Finley et al., 2008, Holden et al., 2013, Aschendorff et al., 2007, Skinner et al., 2007, Dalbert et al., 2016, Wanna et al., 2011). The rate of scalar vestibuli translocation using contemporary electrodes, as diagnosed from post-operative imaging, ranges from 3 – 50%, depending on the electrode type and design (Boyer et al., 2015, Finley et al., 2008, Holden et al., 2013).
Figure 1.7  Cross-sectional histological studies of the cochleae of CI recipients, derived from Lee et al. (2011). Clockwise from top left: fracture of the osseous spiral lamina; displacement of the basilar membrane with the electrode traversing to scala media; dissection in to the lateral wall; fracture of the OSL and translocation of the electrode in to scala vestibuli. Adapted (Lee et al., 2011, pp75-6).

Although not included on the Eshraghi et al. scale, tearing or dissection of the spiral ligament of the lateral cochlear wall has been identified as a point of impingement leading to an incomplete insertion (Lee et al., 2011). Some hypothesised mechanisms of hearing loss from spiral ligament damage include secondary tearing of Reissner’s membrane with intermixing of fluids (Nadol et al., 2001) and basal buckling of the electrode due to the large insertion forces required to overcome the point of impingement (Zhang et al., 2010). A further mechanism is the impairment of stria vascularis function from the occlusion of adjacent vessels. This is believed to result in changes to the cochlear fluids’ ionic composition and endocochlear potential (Wangemann et al., 1995, Tanaka et al., 2014, Reiss et al., 2015).
Microscopic trauma and molecular changes
Since EAS is a relatively recent concept in the history of the cochlear implant, there is currently limited literature on the histopathological changes and propensity for trauma among modern hearing preservation electrodes. Recent studies of contemporary electrodes implanted in preserved cadaveric temporal bones have shown that, in most instances, insertion to a full cochlear turn is achieved without any macroscopic trauma (Mukherjee et al., 2012, Skarzynski and Podskarbi-Fayette, 2010). Animal studies have also shown that post-implantation hearing loss can occur even after an ‘atraumatic’ insertion and in the absence of any gross structural changes (Eshraghi et al., 2005, Lee et al., 2013). Together, these findings have led to a revision of the trauma scale to include microscopic trauma occurring at the molecular level as Grade 0 (Eshraghi and Van de Water, 2006). Several mechanisms of damage at the microscopic or molecular level have been speculated to cause post-implantation hearing loss including: damage to blood vessels at the inner surface of scala tympani (Wright and Roland, 2013); disruption of perilymph (Eshraghi and Van de Water, 2006) and/or lymphatic flow (Wright and Roland, 2013); obstruction of the ductus reunions or cochlear aqueduct (Quesnel et al., 2015).

The blood vessels associated with scala tympani have no bony cover that renders them particularly susceptible to injury (Wright and Roland, 2013). Injury or occlusion of these vessels may adversely affect hair and spiral ganglion cell function (Wright and Roland, 2013), although not yet proven experimentally. Furthermore, larger veins of the basal region, such as the inferior cochlear vein, may be directly damaged with drilling of the cochleostomy (Li et al., 2007b, Guo et al., 2016). This has been shown to cause neuronal losses (Kimura and Perlman, 1956), but whether this is a direct effect of neural injury or exacerbated by intrascalar bleeding is unresolved. Vascular trauma can also result in the intrascalar entry of blood and this has been demonstrated to be especially detrimental to hearing (Radeloff et al., 2007, Ryu et al., 2015), likely by providing a scaffolding for a persistent inflammatory response and later fibrosis (Kel et al., 2013). Damage to perivascular fluid pathways, such as the lymphatic system in the lateral cochlear wall, has also been proposed as a possible cause of residual hearing loss (Wright and Roland, 2013), although not yet proven experimentally.

Intrascalar pressure disturbances that alter neurosensory transduction have been proposed as potential causes of hearing loss at the molecular level. Perilymph is particularly vulnerable to disturbances since it is contained in a sealed compartment with two membranes at either end.
Consequently, any alterations to the membranes (round or oval window) or scalar openings will affect the hydraulic pressure of the compartment. Animal experiments and a study of implant recipients have shown that opening of the RWM (Choudhury et al., 2014, Choudhury et al., 2011, DeMason et al., 2012, Mandala et al., 2012) or creation of a cochleostomy (Radeloff et al., 2007, Mandala et al., 2012, Giordano et al., 2014) results in an immediate, albeit mild, reduction in hearing. Similarly, a rapid reduction in perilymph through suctioning results in a sudden and severe decline in hearing (Mandala et al., 2012, Oshima et al., 2014) with only a partial long-term recovery (Oshima et al., 2014).

Additional fluid disturbances may occur through occlusion of the ductus reuniens or cochlear aqueduct, either from electrode induced trauma or extension of the tissue response. Obstruction of ductus reuniens, a small canal that transmits endolymph between the cochlea and vestibular system, has been found to be associated with endolymphatic ‘hydrops’ in the temporal bones of implant recipients (Richard et al., 2012). The development of hydrops is likely multifactorial since it can also occur without ductus reuniens occlusion (Quesnel et al., 2015, Smeds et al., 2015). While hydrops can be present with a sensorineural hearing loss, such as Meniere’s Syndrome (Merchant et al., 2005), any direct contribution to post-implantation hearing loss is currently speculative. Occlusion of the cochlear aqueduct, a canaliculus that may have a physiological role in perilymph flow (Parnes et al., 1999), has similarly been noted in the temporal bone of an implant recipient with delayed hearing loss (Quesnel et al., 2015). However, the precise role of the cochlear aqueduct (Gopen et al., 1997) and the effect of occlusion on residual hearing also remains uncertain (Quesnel et al., 2015). Other bony channels surrounding the vascular and nerve supplies of the cochlea may provide potential pressure ‘leaks’, however, their high impedance means they are unlikely to influence inner ear pressures substantially (2010).

**Force of insertion**

The force of electrode insertion has been investigated for three primary reasons: improving electrode design (Roland, 2005); providing real time feedback to the surgeon (Miroir et al., 2012); and developing ‘robotic assisted’ surgery (Yang, 2008). ‘Robotic assisted’ surgery that integrates real-time force measurements in to a remote insertion tool are in the very early stages of development (Majdani et al., 2010, Schurzig et al., 2010) and a much larger body of research has been dedicated to improving electrode design, primarily focusing on reducing
frictional forces at the tip and along the length of the electrode. It is widely believed that the magnitude of insertion forces correlates relatively well with the level of intracochlear trauma (Wade et al., 2014). But until recently, correlations between rising force and post-implantation hearing loss had not been shown experimentally (Drouillard et al., 2017).

The peak force of an atraumatic insertion (without gross structural changes) in to cadaveric temporal bones ranges from 60 – 300 mN (Miroir et al., 2012, Rohani et al., 2014, Nguyen et al., 2012). In these instances, the peak in force typically coincides with contact of the outer lateral wall. Interestingly, the peak insertion force required to cause trauma has been found to be incredibly low. An in-situ study with preserved bony attachments found that the force for OSL fracture ranged from 42 – 122 mN (Schuster et al., 2015). Similarly, rupturing of the delicate basilar and Reissner’s membrane required mean forces of only 30mN and 4.2mN, respectively (Ishii et al., 1995). Highly traumatic insertions typically rise to greater than 300mN, such as with scala vestibuli translocation (Rohani et al., 2014) and electrode tip fold-over (Nguyen et al., 2012). The average force over the duration of an insertion, distinct from a single peak in force, ranges from 50 – 200 mN using cadaveric bones (Roland, 2005, Miroir et al., 2012, Rohani et al., 2014, Nguyen et al., 2012). Contour electrodes that conform to the modiolus have been shown to have lower average and peak forces (both less than 100mN) when used with an advance off-stylet technique (Roland, 2005, Schurzig et al., 2010, Majdani et al., 2010) (see 1.4.6 Electrode array designs). While contour electrodes have been demonstrated to result in less intracochlear trauma than straight electrodes (Stover et al., 2005), this result is partly off-set by a heightened risk of incorrect technique with less experienced operators, tip foldover and over-insertion (Cohen et al., 2002) (see 1.4.6 Electrode array designs).

**Modelling of the ideal insertion vector**

Modelling of the ideal insertion vector has been performed by several researchers to investigate the force of insertion and the risk of intracochlear trauma. A knowledge of the ideal insertion vector is especially important for the surgeon since they have very few cues for performing a safe insertion. The surgical field, for example, offers limited visual cues as to the cochlea orientation (Torres et al., 2015) and this can be further restricted by a small facial recess (Roland and Wright, 2006). The surgeon’s range of movement may be also limited by the surgical field, which results in a small margin of error for surgical trauma.
So far, modelling has shown that the linearity of the insertion vector to a centreline axis of the basal turn of scala tympani is directly related to the force exerted against either the OSL, outer wall or modiolus (Roland, 2005, Friedmann et al., 2015). For example, an insertion vector that has excessive modiolar trajectory relative to the centreline axis risks damaging the OSL and Scarpa’s ganglion region, see Figure 1.8A (Meshik et al., 2010, Shapira et al., 2011, Deshpande and Wendell Todd, 2016). A trajectory that is excessively apical in relation to the centreline axis risks damaging the basilar membrane and organ of Corti, see Figure 1.8B (Zhou et al., 2014).

![Figure 1.8](image)

Figure 1.8 Force vector diagram from where electrode first contacts the outer wall in two planes consistent with a cross-section view of the basal turn (A) and a mid-modiolar view (B). Reprinted (Roland 2005, pp20-1).

1.4.6 Implant designs

The fundamental design of the modern electrode consists of platinum stimulating contacts, typically 12 - 22 in number, and lead wires that are encapsulated by silicone (Clark, 2008), see Figure 1.9. Contacts are spread longitudinally along the electrode array to provide stimuli that replicates the cochleotopic arrangement of the normal cochlea (Boyd, 2011). Alterations to this fundamental design include a narrowed diameter (Mukherjee et al., 2012), shortened (Gantz and Turner, 2004) and contoured designs (Briggs et al., 2011).
Figure 1.9  Schematic diagram of an electrode array that has since been released by Cochlear™ on the CI422 implant. The electrode array has 22 half-band electrode contacts over 20mm, approximately a full cochlear turn. Reprinted (Skarzynski et al., p85)

Electrode length
Most patients deemed suitable for hearing preservation CI surgery were initially implanted with a shortened electrode that was limited to the lower basal turn. For example, the Cochlear Ltd Hybrid Implant restricted insertions to 6-10 mm (compared to a cochlear length of 34 mm) and was shown in early studies to preserve hearing (Gantz et al., 2006, Gantz et al., 2005). Because of their limited range within the cochlea, the use of a shortened electrode has the risk of a stimulation mismatch between the presented frequency bands and the cochlear place of stimulation (Briggs et al., 2006, Fu and Shannon, 1999). Additionally, in the event of low frequency hearing loss, these shortened electrodes may provide inadequate low frequency stimulation and this has often necessitated reimplantation with a longer electrode (Fitzgerald et al., 2008). By comparison, when a longer electrode is used, and low frequency subsequently lost, this electrode can simply be reprogrammed without the need for reimplantation and the risk of further hearing loss (Fitzgerald et al., 2008).

Shortened electrodes were initially conceived to reduce the risk of trauma compared with conventional longer electrodes. Longer electrodes were believed to cause a higher accumulative frictional force since they were placed further in towards the apex of the cochlea, where the radius of curvature is higher and the cross-sectional area considerably less (Finley et al., 2008). However, full-length electrodes specifically designed for hearing preservation (18-25 mm in length and extending to the completion of the first turn) have since been demonstrated produce none or minimal gross trauma when implanted in cadaveric human temporal bones (Mukherjee et al., 2012, Skarzynski and Podskarbi-Fayette, 2010). Some low-frequency electrical stimulation can be provided with these electrodes since an electrode depth of 20mm along the lateral wall corresponds to a frequency of approximately 1000 Hz.

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The final depth of insertion, often not accurately measured or reported (Yukawa et al., 2004, Causon et al., 2015), has been found to differ to the electrode’s physical properties (Lee et al., 2011). Unfortunately, this has limited the comparisons of hearing outcomes with insertion depth (Causon et al., 2015). One study of a single electrode designed for a 17 mm insertion depth found that the computed tomography (CT) derived estimations of insertion depth ranged from 11.9 to 25.9 mm (Skinner et al., 2002). Causes of an under-insertion include early irretraceable resistance or cochlear anomalies (Skinner et al., 2002). Studies comparing CT-derived insertion depths with hearing outcomes have been performed on traditional CI subjects (with minimal or no residual hearing) and with mixed results to date. Some studies have shown significant positive correlations between insertion depth and speech recognition (Skinner et al., 2002, Yukawa et al., 2004), while other studies have shown negative correlations (Finley et al., 2008, Holden et al., 2013). These discordant findings may have arisen from electrodes inserted to an unintentional depth. This includes a short electrode inserted deeply into the cochlea that improves electrical stimulation or a traumatic over-insertion of a full-length electrode (Skinner et al., 2002). A further factor is the presence of intracochlear pathology, such as ossification, that may limit the insertion depth as well contribute to a poorer hearing result (Yukawa et al., 2004). Hence, the optimal electrode length, in terms of surgical trauma and preserving residual hearing, has not yet been determined. Based on the current literature, a full-length contemporary EAS electrode should be preferentially used because of the ability to reprogram, instead of reimplanting, in the event residual hearing is lost.

**Electrode array designs**

Electrode array designs used for hearing preservation cochlear implantation surgery have been primarily of two types: straight arrays that tend to track along the lateral wall or contour arrays that position closer to the modiolus (Soda-Merhy et al., 2008). Both designs have several benefits and important limitations over the other. Straight electrodes have been shown in ex-vivo temporal bone studies to have a low risk of causing direct trauma to the osseous spiral lamina or of scalar translocation (Skarzynski and Podskarbi-Fayette, 2010, Mukherjee et al., 2012, Verberne et al., 2016). However, studies of temporal bones and post-operative imaging have found that impacting the lateral cochlear wall can cause upward deflection of the basilar membrane (Adunka and Kiefer, 2006, Finley et al., 2008, Verberne et al., 2016),
which may adversely affect cochlear mechanics and residual hearing (Choi and Oghalai, 2005). Notably, the location at highest traumatic risk is the point of 1\textsuperscript{st} contact between the electrode and the lateral wall, usually at an insertion angle of around 180 degrees (Jolly et al., 2010). Since straight electrodes are intrinsically more flexible at the tip than contour electrodes, because of fewer metallic parts, the risk of trauma in this region is likely to be lower with a straight electrode compared to a linear trajectory of a contour array (Kha et al., 2004).

Contour electrodes are also termed perimodiolar electrodes because of their close location to the modiolus. The advantage of a contour electrode is that the contacts are in proximity to the ganglion cells or, in some cases, surviving peripheral nerve fibres within the osseous spiral lamina. This position requires less electrical current to simulate (Adunka et al., 2006, Rebscher et al., 2008) compared to a straight electrode on the lateral cochlear wall, which can be up to 1 mm from the modiolus (Shepherd et al., 1993). Perimodiolar placement also has a greater likelihood of stimulating a more specific tonoptic region of the cochlea, although it is unclear if this results in better speech perception (Esquia Medina et al., 2013).

One particular concern with contour electrodes is their high rate of scala vestibuli translocation (Aschendorff et al., 2007, Boyer et al., 2015), particularly with a RWM approach since this is dependent on navigation of the curved hook region (Souter et al., 2011). Cochlear’s Contour\textsuperscript{TM} electrodes are usually pre-curved but straightened by a metal stylet. These electrodes were not originally, but are now inserted with a technique called advance off-stylet whereby the stylet is removed while simultaneously inserting the electrode. The performance of the advance off-stylet technique has been found to have variable success among surgeons and has been found to have limited adaptibility for a difficult insertion, such as with early resistance (Zhang et al., 2010). A case comparison study compromising of 61 implantations by a single surgeon performed via a RWM approach found scalar translocation occurred in 8 of 31 (26\%) contour electrode implantations compared to 1 of 30 (3\%) straight electrode implantations (Boyer et al., 2015). A number of centres have reported successful hearing preservation with contour arrays (Cosetti et al., 2013, Carlson et al., 2011), including a single centre study showing comparable results when performed via cochleostomy (Soda-Merhy et al., 2008). In addition, a meta-analysis by Santa Maria et al. (2014) showed no benefit for hearing preservation with either electrode type for combined surgical approach. However, based on anatomical studies and conflicting clinical data, use of a contour electrode

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with RWM approach should be avoided (Friedland and Runge-Samuelson, 2009).

Modern electrode designs have incorporated a softer and smaller diameter tip to reduce friction forces and overall trauma (Adunka et al., 2004). Electrodes that are flexible along their entire length appear to be less traumatic than stiffer arrays, but provide less tactile feedback for the insertion, including when encountering resistance (Jolly et al., 2010). Basal stiffness has thus been added to flexible electrodes to negate this issue through properties built-in to the electrode (Clark, 2008) or trialled experimentally via external components such as a sheath (Briggs et al., 2011). Some straight electrodes have been incorporated with half band ring contacts (Fig …), as opposed to complete rings, which provides a smoother lateral surface and reduces outer wall friction (Lenarz et al., 2006). Thin and flexible electrode arrays have been shown to have lower insertion force profiles, particularly beyond 270 degrees of insertions where the cross-sectional diameter of scala tympani is considerably smaller than in the basal regions (Nguyen et al., 2012). One of the thinnest full-length electrodes in current use is the CI422 made by Cochlear Ltd, which has 22 half-band contacts, measuring 0.3 x 0.35 mm in width (see Figure 1.9).

1.5 Intraoperative monitoring

Presently, feedback of the electrode’s behaviour during CI surgery is limited to only subtle tactile cues and direct visualisation of only the portion of the electrode outside of the cochlea (the ‘extracochlear’ portion). The extracochlear view is further restricted by the structures of the facial recess. Furthermore, extracochlear changes like buckling typically involve large insertion forces and a high probability of trauma (Zhang et al., 2010).

Current feedbacks in clinical practice have very poor responsiveness and sensibilities as to the actual intracochlear behaviour of the electrode (Adunka and Kiefer, 2006), which is highlighted by the relatively frequent occurrence of scalar translocation using contemporary electrodes (Boyer et al., 2015, Finley et al., 2008, Holden et al., 2013). These findings have led to the exploration of alternative methods to monitor the behaviour of the electrode during the insertion. The two real-time measures that have been most investigated are the force of insertion and monitoring the status of residual hearing.
1.5.1 Haptic feedback and force recordings

Tactile cues, termed haptic feedback, have been extensively examined for developing minimally invasive and robotic assisted surgery. Robotic assisted surgery, that integrates force feedback with precise hand movements, have been adopted in many disciplines of surgery (Wheat et al., 2004, Payne et al., 2015). So far, force detection in robotics have had a relatively poor sensitivity (with resolution greater than 100 mN) and bulky configuration that have limited any direct applications in cochlear implantation surgery (Bell et al., 2012, Zhang et al., 2010, Kobler et al., 2014, Hussong et al., 2010).

Haptic forces relating to general uses of the palm of the hand have been found to be dependent upon whether the hand is moving or stationary (Yang, 2008), the weight of what is to be held (Pongrac, 2006), and the angle and area of contact to the palm of the hand (Panarese and Edin, 2011). A standard unit of measurement for weight perception is the Weber fraction, a ratio between the smallest detectable difference and the reference weight. For example, the Weber fraction of a hand-held stylus that is similar in shape to surgical insertion forceps is 0.1 for a stationary hand (Pongrac, 2006) and 0.45 for a moving hand (Yang, 2008). Since the mass of insertion forceps is approximately 15 grams (J Day of Thomas Scientific©, personal communication, October 17, 2016), the range of the smallest detectable differences of handheld forceps, based upon the Weber fraction, is 15 – 65 mN (depending on hand movements). The force required to rupture the basilar membrane is 26 – 35 mN (Ishii et al., 1995), which is often lower than the detectable difference of using handheld forceps and may explain the high rates of trauma using conventional electrodes (Boyer et al., 2015, Finley et al., 2008, Holden et al., 2013).

External systems of force measurement have been investigated to improve upon the relatively crude detectable difference provided by haptic force. The standard method for measuring electrode forces is to place a 6-axis sensor below the specimen, typically a temporal bone or cochlea model in the laboratory. This method is suitable with light-weight isolated specimens, such as a temporal bone, cochlear model or small animal (Drouillard et al., 2017). For example, Drouillard et al. (2017) measured the force of insertion in guinea pigs via a sensor placed under a stereotactic frame and found hearing worsened with more forceful insertions. However, the use of this technique is impractical in the operating room because of the weight of the patient’s head (4.5 – 5 kg, in an adult) and precise positioning on the force
sensor. More clinically applicable methods for force measurements have been shown to be via integration in to the insertion tool or the electrode, itself. Miroir et al. (2012) showed that a uniaxis force sensor, as a component of a custom-made insertion tool, was capable of sensitively (within 4 mN) and accurately recording insertion forces when compared to a standard 6-axis recording placed beneath a temporal bone (Miroir et al., 2012). Similarly, Wade et al. (2014) used an electrode with a built-in uniaxis sensor at its tip to demonstrate a direct correlation between the insertion force and surgical trauma in the guinea pig cochlea. Notably, both studies lacked correlations between intraoperative force and long-term post-implantation hearing loss.

1.5.2 Intraoperative Electrocochleography

Monitoring the status of residual hearing with ECochG during implantation has shown promise for detecting intracochlear trauma and preserving residual low frequency hearing. ECochG is a near-field recording of cochlear neural responses to sound that can be rapidly captured and has been strongly correlated with behavioural responses (see 1.1.5 Measuring the functionality of the auditory system). Importantly, the ECochG waveform captures the various neural responses of the cochlea, including from receptors to nerve cells. For intraoperative recordings, the cochlear microphonic (CM) and the compound action potential (CAP) are the most widely investigated components of the ECochG response.

Clinical studies that preserve the response amplitude of the CAP (Mandala et al., 2012) or CM (Acharya et al., 2016, Campbell et al., 2016) have shown promise in predicting the extent of hearing preservation. The recording configuration for these studies have consisted of either externally placed electrodes (termed, ‘extracochlear’ recordings) or directly from the implanted electrode (‘intracochlear’ recordings) (Campbell et al., 2015, Campbell et al., 2016). Extracochlear recordings require the introduction of a further entity in to a narrow surgical field that can be time consuming for set-up and calibration (Campbell et al., 2015). Conversely, the results of an intracochlear electrode may be confounded by travel within the cochlea as they approach signals generating the response (Campbell et al., 2015). Recent cases of gross surgical trauma, such as kinking of the electrode (Radeloff et al., 2012) and scalar translocation (Dalbert et al., 2016, O'Connell et al., 2017), that coincided with ECochG losses have strongly suggested a role of predicting severe types of surgical trauma. Nevertheless, these ECochG recordings have arisen from observational studies that have
been, at times, volatile (Campbell et al., 2016) and without any correlation to the actual electrode path. Hence, clinical studies alone are yet to provide meaningful interpretations for surgeons performing CI surgery.

Animal studies of intraoperative ECochG recordings have provided useful insights into the electrode path and surgical trauma that occur with changes in the ECochG response. Researchers from North Carolina have shown that irreversible losses to the amplitude of the CM and (to a lesser extent) CAP can be associated with full-thickness damage of the OSL and basilar membrane (Campbell et al., 2010, Choudhury et al., 2011, DeMason et al., 2012). Additionally, reductions in the amplitude of the CM and CAP, from either contact of the basilar membrane or superficial scrapes of the OSL, can be recoverable by retraction of the electrode (DeMason et al., 2012, Campbell et al., 2010, Choudhury et al., 2014). Notably, intraoperative ECochG with findings of the electrode path and long-term hearing loss have not been determined in animal or clinical studies.

1.5.3 Radiological and endoscopic imaging

Radiological imaging, through plain x-ray (Coelho et al., 2008) or CT (Bloom et al., 2009), has been used for diagnosing severe abnormalities of the electrode path, such as kinking, tip fold-over (Coelho et al., 2008), or scalar translocation (Holden et al., 2013). Imaging may therefore assist the surgeon in determining whether to reinsert an electrode array or substitute it for a backup device (Cosetti et al., 2012). However, the poor delineation of fine and soft tissue intra-cochlear structures, such as the basilar membrane, have meant their use at the time of surgery is restricted to when a severe abnormality is suspected. Endoscopic visualisation of the most basal portion of the cochlea has been performed experimentally on animals (Campbell et al., 2010) and humans (Balkany, 1990). But, low image resolution, poor flexibility and large size of the endoscope relative to the intracochlear anatomy have so far prevented their application in CI surgery (Campbell et al., 2010).

1.6 Histopathological response to trauma and the electrode

Direct trauma from surgical access and placement of the electrode creates stimuli that instigates a biological (or ‘histopathological’) response (see 1.4.2 Causes of post-implantation hearing loss). Like direct trauma, the histopathological response can also be
damaging to residual hearing but through several indirect mechanisms. The histopathological response, such as acute inflammation and fibrosis, may cause both immediate and delayed patterns of residual hearing loss (see **1.4.1 Patterns of post-implantation hearing loss**). Immediate or early hearing loss can result from acute inflammation that leads to hair cell death through processes like apoptosis and necrosis (Eshraghi and Van de Water, 2006). Delayed hearing loss may arise from fibrotic changes around the electrode, new bone growth, and a foreign body reaction (Clark et al., 1995, O'Leary et al., 2013) that may damage neural cells directly or dampen cochlear mechanics (Tonndorf and Tabor, 1962). For these reasons, considerable interest has emerged in defining the histopathological response and developing protective strategies that target it (see **1.7 Glucocorticosteroids and pharmacological protection**).

Acute cochlear inflammation responds to ‘danger signalling’ from injured tissue and may last from a few minutes to several days (Kumar et al., 2013). The normal cochlea does not contain large numbers of resident leukocytes (Harris and Ryan, 1995), the cells responsible for inflammation, and relies upon recruitment from the general circulation (Jia et al., 2016, Hirose et al., 2014). Numerous local vascular changes facilitate this recruitment process into the cochlea, including local stasis (Quirk and Seidman, 1995) and increased venule permeability at the modiolus and lateral cochlear wall (Souter et al., 2012). Recruited leukocytes that enter the cochlea, such as macrophages and neutrophils (Kel et al., 2013), become activated in the cochlear fluids and set about resolving the tissue injury (Ma et al., 2000). Activation occurs through pro-inflammatory stimuli, like tumour necrosis factor α. However, a by-product of these inflammatory stimuli is inadvertent damage to the normal cochlear tissue, including hair cells and spiral ganglion cells (Fayad et al., 1991).

So far, implantation studies in animal models have been conflicting about the underlying mechanism for this type of hearing loss, with some demonstrating neural losses (Eshraghi et al., 2013) while others have suggested more subtle changes, such as the hair cell synapse (Reiss et al., 2015). Eshraghi et al. (2013) showed with implanted guinea pigs that inflammatory or immune responses can result in hair cell death and hearing loss. This is proposed to occur through cell death pathways, such as necrosis, necrosis-like programmed cell death and apoptosis (Eshraghi and Van de Water, 2006). Furthermore, inhibitory peptides that target these pathways have been shown to be protective after electrode insertion trauma.
(Eshraghi et al., 2013), but also following prolonged sound trauma and ototoxic aminoglycoside administration (Wang et al., 2003). However, conflicting studies have shown that hearing loss is not explained by hair cell or spiral ganglion cell losses (Tanaka et al., 2014, O'Leary et al., 2013). In a large study of implanted guinea pig cochleae, O’Leary et al. (2013) found that post-implantation hearing loss in response to low frequency stimuli did not correspond to loco-regional hair cell (inner or outer) or spiral ganglion cell losses. Similarly, Reiss et al. (2015) found hearing losses were correlated with vascular changes in the lateral wall secondary to trauma, along with losses in pre- and post-synaptic terminals of hair and spiral ganglion cells, but with no change to hair cell numbers or spiral ganglion cell densities. Overall, these findings suggest the involvement of multiple mechanisms for hearing loss arising from the histopathological response to cochlear implantation.

Another possible cause of post-implantation hearing loss is a longer-term and persistent biological response. Over time, the acute phase of cochlear inflammation is replaced by a chronic phase, characterised by tissue healing and an ongoing inflammatory reaction to the presence of the electrode (Seyyedi and Nadol, 2014). Healing of the cochlea is mediated primarily through macrophages that facilitate the migration and proliferation of fibroblasts to the site of injury (Kumar et al., 2013). These fibroblasts lead to scar formation (O'Leary et al., 2013), which is termed fibrosis due to the abundance of collagen (Kumar et al., 2013). Some level of fibrosis is apparent in all temporal bones of implant recipients (Seyyedi and Nadol, 2014). New bone growth, termed osteoneogenesis, is another prominent feature of the healing process in the cochlea and may occur even without the presence of an overt fracture (Quesnel et al., 2015).

A further component of chronic inflammation is the cellular tissue response. Platinum and silicone, as the principal components of the electrode, are known to be biocompatible with non-irritating and non-sensitising properties. But these electrodes materials are not bioinert, and will still initiate a tissue response that is adherent to its surface (Seyyedi and Nadol, 2014). This tissue response consists of macrophages and their derivatives (epithelioid cells and foreign body giant cells), along with lymphocytes (Kumar et al., 2013). Macrophages are especially common in the tissue response and will coalesce to form foreign body giant cells, as occurs with prostheses elsewhere in the body (Nadol et al., 2014). This tissue response has been found to extend from the point of entry at the cochleostomy or round window, all the way to the tip of the electrode (Seyyedi and Nadol, 2014, Nadol and Eddington, 2004) and

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even beyond (Quesnel et al., 2015). A particularly robust response has been noted at the cochleostomy site (Fayad et al., 2009) and this may relate to the presence of bone dust or blood, intracochlear trauma or the use of a tissue seal to prevent perilymph leakage (Seyyedi and Nadol, 2014, Nadol and Eddington, 2004). While round window insertions have been hypothesised to decrease the severity of a proximal tissue response compared to a cochleostomy (Richard et al., 2012), animal experiments have shown mixed results to date (Rowe et al., 2016, Burghard et al., 2014).

An abundant tissue response on post-mortem analysis has been negatively correlated with the hearing performance of traditional implant recipients prior to death (Nadol et al., 2001, Fayad et al., 2009). This result is somewhat supported in animal experiments, with a (log-)linear relationship between hearing loss and the level of fibrosis above a 40% coverage of the scala tympani (O'Leary et al., 2013). Neurodegeneration from ongoing biological activity (Fayad et al., 2009, Eshraghi et al., 2007a) and dampening of cochlear mechanics (O'Leary et al., 2013, Quesnel et al., 2015) are the proposed mechanisms of post-implantation hearing loss in this setting (1.4.2 Causes of post-implantation hearing loss). A vigorous tissue response has also been noted to increase electrode impedance across the electrode-neural interface (Jia et al., 2016, Wilk et al., 2016), which may reduce electrical hearing performance (Roland and Wright, 2006). Although unrelated to hearing performance, an increased impedance also elevates the level of current required to achieve an electrical stimulus and this leads to a shortened battery life (Clark et al., 1995).

1.7 Glucocorticosteroids and pharmacological protection

So far, several interventions aimed at reducing or negating the histopathological response to CI surgery have been investigated. These include pharmacological agents like n-acetyl cysteine (Eastwood et al., 2010), along with preliminary work on gene therapy and anti-apoptotic agents (Jia et al., 2013), such as peptide inhibitors targeting cell death pathways (Eshraghi et al., 2013) and neurotrophins (Sly et al., 2012). However, the most investigated and promising intervention to date is glucocorticosteroids, a group of steroid hormones produced in response to various types of physical and mental stress (Brenner and Stevens, 2013).
1.7.1 Mechanism of action

Glucocorticosteroids have profound anti-inflammatory and immunosuppressive actions (Bas et al., 2012), which occur via two pathways: non-genomic and genomic expression. The non-genomic pathway occurs rapidly in seconds to minutes via interactions at the cell membrane surface (Losel and Wehling, 2003). The effects of the non-genomic pathway include disruption of cellular processes that lead to apoptosis (Losel and Wehling, 2003, Bas et al., 2012) and diminishing (or preventing) the acute immune response by interfering with processes such as the intracellular calcium concentration (Buttgereit et al., 1999).

Genomic effects occur by the modulation of gene transcription (Buttgereit et al., 1999) and protein synthesis. Since some of the mechanisms of action of are dependent on protein synthesis, the effective (or biological) half-life is far more prolonged than the plasma half-life (Brenner and Stevens, 2013). As an example, dexamethasone is a synthetic glucocorticosteroid that has a prolonged action of up to 2 days but a far shorter plasma half-life of approximately 180 minutes (Brenner and Stevens, 2013). Some genomic effects include inhibiting the release of arachidonic acid, a secondary messenger and inflammatory intermediary that contributes to every step of the inflammation pathway (Kumar et al., 2013). Glucocorticosteroids dramatically inhibit the exudation of plasma and reduce the accumulation of leukocytes to the site of inflammation (Boumpas et al., 1993). Additionally, they can inhibit fibroblast growth and reduce the overall extent of fibrosis (Boumpas et al., 1993). Since glucocorticosteroids have a broad range of actions on different cell types, the target tissue response may vary depending on the cell population and their physiological properties (Boumpas et al., 1993). In the human cochlea, glucocorticosteroid receptors are found with highest distribution in proximity to the organ of Corti, spiral ligament and stria vascularis (Terunuma et al., 2001).

1.7.2 Medical uses of glucocorticosteroids

Glucocorticosteroids are currently used in the treatment of a range of inflammatory, allergic and immunologic disorders. This includes the use of glucocorticosteroids on organs such as the liver, where they have been demonstrated to reduce hepatic fibrosis (Dufour et al., 1997), and with cardiac pacemaker leads, in which decreased impedance and threshold are apparent (Mond and Stokes, 1996). Glucocorticosteroids have also been used for several inner ear
diseases (Garduno-Anaya et al., 2005, Xenellis et al., 2006). For example, a randomised trial of vestibular neuritis, an inflammatory inner ear condition, showed significantly improved recovery from symptoms with glucocorticosteroid treatment (Strupp et al., 2004). Glucocorticosteroids have also been traditionally used as first-line treatment for patients with idiopathic sudden sensorineural hearing loss (Parnes et al., 1999), however, this was shown to have conflicting outcomes among randomised controlled trials (Wei et al., 2013).

Some of the common undesirable effects of glucocorticosteroids include tissue and metabolic changes that usually occur after prolonged therapy of more than 2 weeks (Brenner and Stevens, 2013). A variety of synthetic glucocorticosteroids also have overlapping binding of the mineralocorticosteroid receptor that influences salt and water balance. Mineralocorticosteroid side effects may include fluid retention, although, the extent of which is largely dependent on the drug type, dosage and duration (Brenner and Stevens, 2013). For example, prednisolone and methylprednisolone have some mineralocorticosteroid potency while dexamethasone and triamcinolone have none or negligible potency (Brenner and Stevens, 2013).

1.7.3 Glucocorticosteroids and cochlear implantation

Among several small and low-powered clinical studies, glucocorticosteroids have shown promise for preserving hearing and reducing electrode impedance (Carlson et al., 2011, Enticott et al., 2011, Kuthubutheen et al., 2012, Paasche et al., 2009, Rajan et al., 2012, Sweeney et al., 2015, Cho et al., 2016). For example, Cho et al. (2016) found a combination of glucocorticosteroids delivered systemically and topically at various time intervals resulted in significantly greater hearing preservation rates than with control patients not receiving any steroid. Inherent weaknesses for these clinical studies have been the absence of randomisation, a small study size and inconsistent use of any single implant or surgical approach. More recently, a small but randomised study using a single implant demonstrated a transient improvement in residual hearing with topical therapy compared to placebo (Kuthubutheen et al., 2017). However, no treatment effect was found with systemic glucocorticosteroid administration or at the final 12-month time-point for either treatment groups (Kuthubutheen et al., 2017). Two meta-analyses have reported improvements in hearing outcomes following glucocorticosteroid administration, albeit with conflicting results on the optimal mode of delivery (Santa Maria et al., 2014, Causon et al., 2015). These meta-
analyses also included various dosing regimens, such as systemic treatment given before (Bruce et al., 2011, Kuthubutheen et al., 2012), during (Kiefer et al., 2004, Carlson et al., 2011) or after surgery (James et al., 2005, Skarzynski et al., 2007, Usami et al., 2011) and topical treatment delivered via transtympanic means (Gstoettner et al., 2009, Arnoldner et al., 2011) or injected into the middle ear (Jayawarden et al., 2012, Kuthubutheen et al., 2012).

Mode of delivery
The mode of glucocorticosteroid delivery is an important consideration since topical and systemic preparations have distinct differences in their penetration of cochlear tissue and fluids (Parnes et al., 1999). Since glucocorticosteroids are permeable to the blood-labyrinthine barrier, systemic delivery has been shown to result in similar concentrations between cochlear tissue and blood plasma (Parnes et al., 1999, Tobita et al., 2002). Entry into the perilymph requires systemically delivered glucocorticosteroids to pass down a very large concentration gradient, thus leading to a lower perilymph level than what is achieved with local delivery (Parnes et al., 1999). However, locally delivered glucocorticosteroids have several disadvantages. These include poor suppression of systemic inflammatory cells (Bird et al., 2011), slow diffusion across the round window membrane (if delivered by this approach) (Chang et al., 2009) and a large concentration gradient between basal (the site of application) and apical regions (the site of residual hearing) (Plontke et al., 2008). Notably, similar rates of hair cell uptake have been identified between locally administered and high-dose systemic glucocorticosteroids (Grewal et al., 2013).

Drug-eluting electrodes, developed to release infused glucocorticosteroids nearby in to the cochlear fluids, have been used for many years in cardiac pacemaker leads for improving cardiac stimulation (Groothuis et al., 2014). Animal studies of drug-eluting electrodes have shown promise for reducing fibrosis tissue growth (Wrzeszcz et al., 2014, Wilk et al., 2016) and electrode impedance (Wilk et al., 2016). So far, clinical studies have been somewhat delayed by the need to accurately determine drug release profiles (Douchement et al., 2014) and the risk of damage to cochlear soft tissue and bone (Niedermeier et al., 2012). In summary, local and systemic glucocorticosteroid treatments provide very different perilymph and cochlear fluid concentrations, however, with the potential for similar hair cell uptake.

Treatment efficacy in animals

Chapter 1: Introduction to cochlear implantation and trauma
In animal studies that compare systemic administration of glucocorticosteroids, reductions in post-implantation hearing loss have been largely demonstrated. This includes generalised (Quesnel et al., 2011, Lee et al., 2013), incomplete or transient improvements in hearing (Connolly et al., 2011, Rah et al., 2016), and with reductions in the tissue response (Connolly et al., 2011, Rah et al., 2016, Lee et al., 2013). On the other hand, local delivery has shown only modest effects on hearing (Lee et al., 2013, James et al., 2008) and with no (Stathopoulos et al., 2014, Braun et al., 2011, Chang et al., 2009) or limited effect (Lee et al., 2013, James et al., 2008, Wilk et al., 2016, Farhadi et al., 2013, Jia et al., 2016, Wrzeszcz et al., 2014) on the tissue response occupying scala tympani. Across all studies, the extent of intracochlear scarring and foreign body reaction has varied from minimal (Stathopoulos et al., 2014) to near complete obliteration of the scalae. Notably, complete obliteration of the scalae may (Lee et al., 2013) or may not be (Connolly et al., 2011, Kuthubutheen et al., 2015, Rah et al., 2016) associated with significant surgical trauma and this may explain the variability identified with the efficacy of glucocorticosteroids.

1.8 Thesis Overview

Post-implantation hearing loss occurs from direct surgical trauma and from the resultant histopathological response. But with such marked differences between hearing loss, trauma and electrode designs, it has been difficult to understand precisely how surgical trauma contributes to hearing loss and more importantly, whether this applies to human cochlear implantation also. The absence of a standardised implantation model has further hindered the investigation of post-implantation hearing loss, along with treatments and monitoring techniques for successful hearing preservation cochlear implantation surgery.

Chapter 2 outlines the equipment and techniques used in the thesis. Experiments were performed on guinea pigs under general anaesthesia due to the invasive surgery needed to access the cochlea. Chapter 3 investigates a cochlear implantation animal model through the use of a novel imaging technique. The risks and variabilities of direct and indirect cochlear damage were determined for a range of surgical approaches and electrode insertion profiles, with comparisons made to human cochlear implantation surgery. Chapter 4 and 5 explore the determinants of cochlear injury in a standardised animal model, how they relate to post-implantation hearing loss, and whether glucocorticosteroids and intraoperative monitoring can prevent these losses. Chapter 6 provides a summary and a general discussion of the
results that includes clinical implications and future directions of this work.

1.9 Aims and Objectives

The principal aim of the studies described in this thesis is:

To examine the different types of cochlear trauma, how they relate to hearing loss and how hearing loss can be prevented

Based upon the results attained by previous studies, the central hypothesis of this thesis is that specific pathologies arising from surgery influence post-implantation hearing loss, along with the amount of hearing preservation achieved with systemic glucocorticosteroids and intraoperative monitoring. The current thesis was therefore undertaken with the purpose of:

i. Determining the role of surgical approach and electrode insertion vector on trauma from cochlear implantation surgery in guinea pigs

ii. Examining the influence of insertion depth on surgical trauma, post-implantation hearing loss and the efficacy of systemic glucocorticosteroids

iii. Examining the influence of electrode flexibility on surgical trauma, post-implantation hearing loss and glucocorticosteroid efficacy

iv. Determining the effectiveness of intraoperative force and electrophysiology to predict surgical trauma
Chapter 2: Materials and Methods
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2.1 Overview

The following chapter describes the methodological techniques utilised in our investigation of trauma and post-implantation hearing loss. This outline of in vivo electrophysiology and imaging techniques applies to all three results chapters. All procedures performed during the course of this study were carried out in accordance with the Royal Victorian Eye and Ear Hospital’s Animal Research Ethics Committee guidelines (projects 14/299AU and 15/335AU) and adhered to the guidelines of the National Health and Medical Research Council (NHMRC) of Australia.

2.2 Animal care and ethics

A total of 86 adult Dunkin-Hartley tricolour guinea pigs, weighing more than 400 g, were used in this thesis. The animals were primarily bred and housed in the Biological Research Centre, at the Department of Otolaryngology (The University of Melbourne, Royal Victorian Eye and Ear Hospital). In addition, a small number of animals were bred and housed at the Bionics Institute (Melbourne), and imported to the department on the day of experiments.

2.2.1 Anaesthesia and surgical preparation

All guinea pigs were anaesthetised according to the Royal Victorian Eye and Ear Hospital’s Animal Research Ethics Committee’s Standard Operating Procedure No: 11 (‘Guinea Pigs – Anaesthesia, Pre and Post-Operative Care’). Anaesthesia was inducted through the administration of intramuscular ketamine (60 mg/kg, Troy Laboratories Pty Ltd, Sydney, Australia) and xylazine (4 mg/kg, Troy Laboratories Pty Ltd). Maintenance doses were administered every hour (40 mg/kg of ketamine and 4 mg/kg of xylazine). Additional doses were given upon observation of either corneal reflexes, or paw-pincht hindlimb withdrawal at any further time during the course of the experiment. Whilst under anaesthesia and before any skin incisions, a total of 0.5 ml of the local anaesthetic lignocaine 2% (Xylocaine®; AstraZeneca, Macclesfield, Cheshire, UK) was administered. Paw or skin reflexes observed
during the initial incision of the skin resulted in the administration of a small supplemental dose of 2% lignocaine.

Upon attaining deep anaesthesia, the neck and left post-auricular region of the animal was shaved. Ocular lubricant was applied (and repeated as required) to prevent corneal drying. The animal’s ear canals were inspected with a microscope in order to examine the integrity of the tympanic membrane. The animal was wrapped in a towel to minimise heat loss and positioned in partial right lateral decubitus, for best orientation of the middle ear and cochlea during implantation. The animal was housed in an anechoic (sound-proof) chamber throughout all surgical and recording stages of the experiment.

Post-operative analgesia was administered in the form of subcutaneous buprenorphine (0.03 mg/kg) as per the Standard Operating Procedure No: 11 (‘Guinea Pigs – Anaesthesia, Pre and Post-Operative Care’). Following the procedure, animals were placed in a recovery box, kept warm and monitored by the researchers until mobile. The animals were then moved to a housing box and monitored further to ensure they were eating and drinking.

2.3 Surgery

All animals underwent a left sided cochlear implantation in Chapter’s 4 and 5, after receiving either intravenous dexamethasone (‘steroid’) or saline (‘control’) 60 minutes prior. Extracochlear ECochG recordings were performed before surgery and at the survival endpoint of 4-weeks. One surgeon performed all surgeries and was blinded to the intravenous treatment and electrode type. All researchers were blinded to the treatment groups for hearing and anatomical analysis.

2.3.1 Intravenous steroid administration

Delivery of the intravenous agent (dexamethasone or saline) was performed 60 minutes prior to the cochleostomy. It was performed in supine position via a surgical cut-down approach on the right internal jugular vein. After local anaesthetic administration, a small horizontal incision was made over the right internal jugular vein in the anterior neck, contralateral to the side of implantation. The internal jugular vein was dissected from surrounding tissue and the distal portion ligated. A small venotomy was performed, followed by placement of a 25-
gauge cannula into the internal jugular vein and a ligature placed proximally, over the cannula. The intravenous agent had a total intravenous volume of 1 ml per kilogram of body weight and was slowly injected over 30 seconds before the cannula was removed and the proximal ligature tied off. Skin closure was achieved with superglue (UHU, GmbH, Bühl, Germany).

2.3.2 Technique for cochlear implantation

Following local anaesthetic administration, a curvilinear incision in the shaved left postauricular region was made with a 15-blade scalpel through the skin and subcutaneous tissues (Figure 2.1). A self-retraining retractor was then placed into the wound to aid access. The muscular attachments to the bulla were retracted inferiorly, giving exposure to the auditory bulla. Using an operating microscope, a bullostomy was performed with a 3 mm cutting burr (Chapter 4) or with a scalpel (Chapter 5). A scalpel was used for the later study to create a larger bullostomy that would accommodate the intraoperative monitoring apparatus. Care was taken not to interfere with the ossicles or external auditory canal from either use of the drill or scalpel.

![Figure 2.1](image.png)

**Figure 2.1** Images from guinea pig cochlear implantation surgery: (A) Protection of the left guinea pig, revealing the site of the post-auricular incision. (B) Muscular attachments to the bulla have been retracted and a small bullostomy performed. (C) Microscopic view of the left cochlea through the bullostomy with a cochleostomy of the basal turn of the cochlea.

Under magnification, a 0.8 mm diamond burr was used to place a cochleostomy approximately one width of the drill bit from the RWM in an anteroinferior plane. Flushing
away of visible bone dust was performed and direct suctioning of perilymph was avoided. The cochlear implant was passed at a constant slow speed (10-15 mm/min) into the cochlea to the depth designated by the silastic plug or until first resistance was met, whichever came first. The implant was secured proximally to the edge of the bullostomy with carboxylate cement (DurelonTM, 3M ESPE AG, Seefeld, Germany), while avoiding the entry of cement into the middle ear.

The muscle over the bulla and skin was closed and sutured with 3-0 coated vicryl (polyglactin suture; Ethicon, Johnston & Johnston Medical Pty Ltd, Australia). The wound was then cleaned with sterile water and lightly sprayed with water-resistant dressing spray Opsite (Smith & Nephew, Medica Limited, U.K.).

2.3.3 Electrocochleography recordings

ECochG was recorded with a 0.5mm diameter gold ball electrode placed directly on the RWM and a subcutaneous needle electrode placed on the nape. An initial extracochlear ECochG recordings was performed prior to cochleostomy and a final recording was made at the end of the 4-week survival period. For final recordings, animals were again anaesthetised, and their wounds reopened with scalpel after local anaesthetic administration. Care was taken not to interfere with the ossicles or external auditory canal and the gold ball electrode was again placed on the RWM to facilitate final recordings. After final recordings, all animals were euthanized for tissue harvesting.

2.4 Perfusion and tissue processing

Upon completion of all surgery and hearing tests, animals were deeply anaesthetised and euthanized according to Standard Operating Procedure No: 2 (Euthanasia in Rats, Mice & Guinea Pigs). Animals were administered an overdose of intraperitoneal pentobarbitone sodium (1.0 g/kg, Lethobarb®; Virbac, Peakhurst, NSW, Australia). After respiratory arrest, a transcardiac perfusion with phosphate-buffered normal saline solution was performed. In this procedure, a midline incision was made on the ventral surface of the chest, to enable access to the heart. The right ventricle of the heart was opened with scissors to allow exsanguination. A 22-gauge catheter was then inserted in to the left ventricle, followed by administration of approximately 100-150 ml of 0.9% phosphate buffered saline for complete
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Exsanguination. This was followed by 10% formalin solution perfused under pressure to promote tissue fixation. The animals were then decapitated, and the cochleae harvested and submerged in 10% formalin for 48 hours.

Cochleae were decalcified in 10% (w/v) ethylenediaminetetra-acetic acid for 3 weeks. Cochleae were then dehydrated by immersion in a graded ethanol series (50%, 70%, 90%, 90%, 100%, and 100%) to remove all water from the sample and then immersed in hexane. Each specimen was cleared to transparency (to reduce light scatter in the tissue) through 48 hours immersion in Spalteholz fluid (5:3 of methyl salicylate: benzyl benzoate; Sigma-Aldrich, USA). Staining or fluorescent techniques were avoided because of possible distortion of the intracochlear anatomy with thin sheet laser imaging microscopy.

2.5 Thin sheet laser imaging microscopy (TSLIM)

The TSLIM device consisted of a 400 mW green (λ = 532 nm) LASER illuminator (OEM 400 mW RGB Laser Chassis, OdicForce Lasers) mounted on a horizontal optical bench rail (Figure 2.2). A 532 nm laser was selected as it stimulates fluorescence of a wide variety of biological structures (Axelsson, 1968). The beam was first passed through a Galilean beam-expander followed by a beam-splitter that created two beam-lines. The two beam lines were guided to opposite sides of a custom-designed specimen chamber, consisting of bonded microscope coverslips with an open top and filled with Spalteholz fluid. A cylindrical lens, an aperture, and x 10 microscope objective were used to focus each laser beam into a thin collimated sheet in a vertical plane. The LASER beams were positioned from opposite sides and orthogonal to the optical axis of a horizontally mounted Olympus MVX4 microscope (Olympus America, Inc., Center Valley, PA) that was attached to a Canon EOS 70D camera (Canon, Tokyo, Japan). Each cochlea was attached to a wooden rod that was placed in the middle of the specimen chamber and connected to three micropositioners (Thorlabs Inc., Newton, NJ), enabling x, y, z translations.
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Figure 2.2  TSLIM on a perforated hardboard, showing the experimental (left) and schematic setup (right). A green LASER beam (1) is expanded (2) and then split (3). Mirrors (4) are used to reflect one beam to the opposite site. Each beam is transformed to a thin light sheet after passing through a cylindrical lens (5) and an illuminating objective (6). The cochlea is moved through the light-sheet in XYZ directions via micropositioners above the specimen chamber (7). The image is recorded with a microscope objective (8) and camera (9). Partially in frame in the experimental setup are the micropositioners and related apparatus from which the specimen was suspended.

Images were taken with the cochlea positioned at the intersection of the LASER sheets. Two column (y) and two row (x) images of optimal resolution were acquired at intervals of 10 µm in the z axis, totalling approximately 1,600 images per cochlea. Control of the micropositioners was automated using a custom LabVIEW program (version 8.6; National Instruments, Austin, TX) and image stitching of the four cross-sectional images into a composite image was automated with MatLab (version 15b, Mathworks Inc., Natick, MA). The z stack of stitched images was imported into Amira 5.4 (Visualization Sciences Group, France) for image analysis.

2.5.1 Post-experiment analysis of implanted cochleae

Detailed visual inspection of the lower basal turn of the cochlea, along with volume calculations of tissue reaction were performed using Amira 5.4 (Visualization Sciences Group, France). The implant’s position was inferred from its impression on the tissue reaction, typified by a circumferential or semilunar space in the tissue. To estimate the extent
and location of tissue response to the electrode, relevant voxels were labelled using Amira’s Segmentation Editor beginning from the cochleostomy. Voxels were selected using the paintbrush on every 3rd slice and then interpolated for intervening slices. The resulting volume was allocated to a new limit. The limits of the scala tympani, where the electrode was present, were similarly labelled, interpolated and saved as a new material. Volumes of each material were calculated by Amira through interpolation between area measurements on sections and using the known distance between sections (10 µm).

To better explore the intracochlear pathology, electrode path and loco-regional tissue response, further analysis was performed with cross-sectional slices. The cross-sectional slices used a plane of rotation with modiolus at its centre, and with a zero-reference angle from the midpoint of the RWM since it is in close approximation to the most basal region of the organ of Corti (Verbist et al., 2010). Tissue response and the path of the electrode were then determined for every 30 degrees from the zero-reference angle in a plane of rotation with the modiolus at its centre. Labelling of the tissue response and scala tympani was performed to calculate a proportion of scala tympani occluded by the tissue response. The level of trauma was graded by visual inspection as per the scale reported by Eshraghi et al. (2006): 1 - no observable macroscopic trauma; 2 - elevation of the basilar membrane; 3 - translocation of electrode to scala vestibuli; 4 - fracture of the osseous spiral lamina (OSL) or modiolus (see Table 1.3). OSL or modiolus fracture was defined as a break in the internal lamina, with or without the presence of osteoneogenesis. Traumatic insertions were deemed to have scored >1 on the Eshraghi scale in any cochlear region. An angular depth of insertion was determined as degrees of rotation around the modiolus, commencing at the midpoint of the RWM and finishing at the electrode tip. This was determined by assuming an axial orientation on Amira, defined as a plane normal to the modiolus (Xu et al., 2000), and using the 2D angle tool.

Amira’s spline tool was used to determine intracochlear distances. A cochleotopic map was created by passing a spline along the tunnel of Corti, from the helicotrema to the round window. This length was mapped to Wada’s equations (Wada et al., 1998), which was adapted from Greenwood’s guinea pig work (Greenwood, 1990). Figure 2.3 shows the Greenwood formula for determining the distance from the apex (in mm) for a given frequency (F). Table 2.1 shows the anatomical based frequency place mapping, in terms of distance from the apex and round window, of pure tones tested in this thesis. Based on the

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frequency place map for guinea pigs (Greenwood, 1990), the 32 and 24 kHz frequency responses originate from the lower basal, the 16 and 8 kHz responses from the upper basal and the 2 kHz response from the lower second turn.

\[
F = 350 \times \left(10^{\frac{2.1 \times \text{dist from apex (mm)}}{18.5}} - 0.85\right)
\]

\[
\text{dist from apex (mm)} = \frac{\log_{10} \left(\frac{F}{350}\right) + 0.85}{2.1/18.5}
\]

**Figure 2.3** An equation for determining the distance from the apex (in mm) of a given frequency (F) for the guinea pig. Adapted from “A cochlear frequency-position function for several species--29 years later,” by Greenwood DD, 1990, J Acoust Soc Am, Vol 87, pp 2597-9.

<table>
<thead>
<tr>
<th>Cochlear frequency (kHz)</th>
<th>Distance from apex (mm)</th>
<th>Distance from RW (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.2</td>
<td>11.3</td>
</tr>
<tr>
<td>4</td>
<td>9.6</td>
<td>8.9</td>
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<tr>
<td>8</td>
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<td>14.7</td>
<td>3.8</td>
</tr>
<tr>
<td>24</td>
<td>16.2</td>
<td>2.3</td>
</tr>
<tr>
<td>32</td>
<td>17.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Table 2.1** The anatomical distance (from the apex and round window) based upon the frequency-place mapping for pure tones that were tested in this thesis (Greenwood, 1990).

Amira’s spline tool was also used to determine the distance of the cochleostomy from the round window. The midpoint of the round window and cochleostomy were both determined by the intersection of two perpendicular views. The Amira spline tool was then used and
2.6 Testing of hearing

Hearing in Chapter’s 4 and 5 was tested as extracochlear ECochG recordings in response to tone pips performed before surgery and at the survival end-point of 4-weeks. ECochG was recorded with a 0.5mm diameter gold ball electrode (provided courtesy of Sjaak Klis, University Hospital Utrecht, Netherlands) placed directly on the RWM and from a subcutaneous needle electrode placed on the nape. Threshold shift was defined as the difference between the pre-operative threshold and the post-operative threshold. Therefore, a positive threshold shift indicated an elevation of auditory thresholds.

2.6.1 Acoustic stimuli

Acoustic stimuli were delivered via a loudspeaker (Richard Allen DT-20, Portsmouth, UK) placed 0.1m from the test ear. The contralateral ear was occluded using an ear mould compound (Otoform K2, DLT, West Yorkshire). The frequency-specific cross-head attenuation, measured from the occluded ear canal of the guinea pig using the same recording configuration, is shown in Table 2.2. The stimuli for ECochG recordings were computer generated tone pips of 20 ms duration with 1 ms linear onset/offset ramps, presented at 2, 4, 8, 16, 24, and 32 kHz. These were presented every 50 ms and averaged over 25 repetitions.

<table>
<thead>
<tr>
<th>Cochlear frequency (kHz)</th>
<th>Frequency-specific cross head attenuation (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>32</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 2.2 Frequency-specific cross-head attenuation, measured from the occluded ear canal of the guinea pig. Adapted from “Delayed low frequency hearing loss caused by cochlear implantation interventions via the round window but not cochleostomy,” by Rowe D, 2016, Hear Res, Vol 333, p50.
2.6.2 Recording configurations

Responses were preamplified by a factor of 1,000 (DAM-5A, W-P Instruments Inc., New Haven, CT) and then band-pass filtered (Krohn-Hite 3750) at 0.1 Hz to 100,000 kHz. The responses were digitized by a 24-bit analogue-to-digital converter (DT9847S) that sampled at 216 kHz. Alternating stimuli with condensation and rarefaction phases were saved separately, allowing them to be added to derive a compound action potential (CAP). CAP traces were then digitally filtered between 10 and 1500 Hz. Hearing thresholds were visually inspected in 5 dB decrements and determined as corresponding to the minimal intensity for which a CAP was clearly recognized (Quesnel et al., 2011). The maximum output plus 5 dB was recorded if no responses were present to the maximum output (Dalbert et al., 2015).

2.7 Animal cochlear implant electrodes

The underlying electrode array design has previously been described (Connolly et al., 2011, Lee et al., 2013). The cochlear implant was an electrode array that was 15mm in length. A number of platinum rings were welded to its tip (three rings in Chapter 4 and one in Chapter 5). Each platinum ring was connected to an internal platinum wire that was 25 µm in diameter, which was helical or straight, depending on the study design (helical in Chapter 4, straight in Chapter 5). All parts were embedded in a silastic carrier, although the type of silicone used was also altered depending on the study design. The maximum diameter of the implant was 0.45 mm, narrowing to 0.41mm towards the tip, which models the tapering of contemporary human electrode designs for a similar tactile response (Rebscher et al., 2007).

Efficacy of preoperative steroids study

A small silastic plug, designed to restrict the depth of insertion, was placed 1.5, 3.0 and 5.0 mm from the tip of the electrode in Chapter 4. Each implant had three platinum rings at its tip, welded 0.75mm apart. Each platinum ring was connected to an internal platinum wire that was 25 µm in diameter and tightly coiled as a helix to resemble contemporary human electrode designs (Rebscher et al., 2007). All parts were embedded in a silastic carrier comprised of a single type of silicone (MDX4–4210 Dow Corning Products, USA).
**Intraoperative monitoring study**

The cochlear implant array for Chapter 5 consisted of a single platinum ring at its tip, welded to a straight platinum 25 µm in diameter. A straight (rather than helical) platinum wire was used so that stiffness could be reliably altered. The ring and internal wire were embedded in a silastic carrier comprised of one of two types of medical-grade silicone: hard (MDX4-4880 Dow Corning Products, USA) or soft (MDX4-4210 Dow Corning Products USA). Other modifications included a platinum contact running perpendicular to the array, located at the basal end of the electrode and welded to the internal platinum wire. This enabled intracochlear ECochG recordings from the ring electrode at the tip of the array to be conducted to the platinum contact that remained outside of the cochlea. The justification for these changes are discussed in **5.3.1 Cochlear implant array – addendum**.

**2.8 Statistical analysis**

Statistical analyses for hearing, force and TSLIM results were performed with IBM SPSS version 23.0 (IBM Corp., Armonk, NY). Specific statistical tests are detailed in the study methodology.
Chapter 3: Hook Region Anatomy of the Guinea Pig Cochlea
Chapter 3: **Hook Region Anatomy of the Guinea Pig Cochlea**

### 3.1 Introduction

Successful hearing preservation cochlear implantation surgery is widely believed to be dependent upon limiting irreversible cochlea injury from direct trauma and the resultant biological response (or ‘indirect’ damage). However, the precise contributions of direct and indirect cochlear damage to post-implantation hearing loss remain indeterminate, in part, because of the lack of a reproducible animal implantation model. Animal experimentations have been partly hampered by the difficult access to the mammalian inner ear. For example, adult mice are small and tedious to operate on, with a weight range of between 20 and 30 g, while rats have a stapedial artery overlying the otic capsule that is prone to injury during experimental cochlear implantation surgery (Pawlowski et al., 2011).

The aim of this study was to determine the reliability and applicability of the guinea pig cochlea for the modelling of hearing preservation cochlear implantation surgery. The guinea pig cochlea is one of the most investigated animal models owing to an ease of access (Rebscher et al., 2007). However, studies investigating CI in guinea pigs have shown marked differences in trauma (O'Leary et al., 2013, Farhadi et al., 2013, Honeder et al., 2015) and resultant tissue response, ranging from minimal (Stathopoulos et al., 2014, Lee et al., 2013, Wilk et al., 2016) to almost total occlusion of the scala tympani of the lower basal turn (Smeds et al., 2015, Wrzeszcz et al., 2014, James et al., 2008, Rah et al., 2016, Braun et al., 2011, Lee et al., 2015).

Techniques performed by our Department, alone, have included implantation via the RWM (Rowe et al., 2016) or via a cochleostomy. Cochleostomies have been placed in an inferior (Smeds et al., 2015) or anteroinferior plane (James et al., 2008), and near to (Lee et al., 2013) or some distance from the RWM (Connolly et al., 2011). Hence, we set out to define the anatomy of the guinea pig hook region and lower basal turn so that the variability in implantation and resultant trauma could be avoided. The study also set out to determine the structural similarities and differences to the human cochlea and how this relates to cochlear implantation surgery. An anteroinferior placed cochleostomy has been shown in human

*Chapter 3: Hook region anatomy of the guinea pig*
anatomical and ex vivo studies to be the safest location (Li et al., 2007b, Guo et al., 2016, Briggs et al., 2005, Adunka and Buchman, 2007) and a particular research question was whether the same applied for the guinea pig cochlea.

To achieve the specific aims of this study, a whole-specimen imaging technique was required. Micro-computed tomography (micro-CT), a whole-specimen analysis, has been previously used by our Department (Smeds et al., 2015). However, the primary use of micro-CT is for imaging bone, rather than soft tissue, because of the use of x-rays reliant on radiodensity. TSLIM is a lesser known whole-specimen technique that optically sections using laser light. TSLIM does require additional steps of decalcification and clearing but has been shown to produce high-resolution images of both soft and bony tissues of non-implanted cochleae (Buytaert et al., 2013). Hence, TSLIM was developed and refined by the Department to address the specific aims of this study.

The hypotheses of this study were that:

i) An anteroinferior placed cochleostomy has the least variability and risk of trauma from drilling and inserting a straight electrode

ii) A cochleostomy positioned furthest from the round window has the least variability and risk of trauma from drilling and inserting a straight electrode

3.2 Otology & Neurotology Publication
Defining the Hook Region Anatomy of the Guinea Pig Cochlea for Modeling of Inner Ear Surgery


*Department of Otolaryngology, University of Melbourne; and †Royal Victorian Eye and Ear Hospital, East Melbourne, Victoria, Australia

Hypothesis: The aim of this study was to describe the hook region anatomy of the guinea pig cochlea to identify the optimal surgical approach for cochlear implantation and to determine what anatomical structures are at risk.

Background: Animal studies investigating hearing loss after cochlear implantation surgery are currently constrained by the lack of a reproducible implantation model.

Methods: Guinea pig cochleae were imaged using thin-sheet laser imaging microscopy. Images were stitched, reconstructed, and segmented for analysis. Insertion vectors were determined by tracing their paths to the outer wall and converting to Cartesian coordinates. Spherical surface and multiplane views were generated to analyze outer wall and radial forces of the insertion vector.

Results: Thin-sheet laser imaging microscopy enabled quantitative, whole specimen analysis of the soft and bony tissue relationships of the complex cochlear hook region in any desired plane without loss of image quality. Round window or cochleostomy approaches in the anteroinferior plane avoided direct damage to cochlear structures. Cochleostomy approach had large interindividual variability of angular depth and outer wall forces but predictable radial force.

Conclusion: The guinea pig hook region and lower basal turn have similar structural relationships to humans. Careful cochleostomy placement is essentially for minimizing cochlear trauma and for ensuring a straight insertion vector that successfully advances around the outer wall. Experiments with guinea pigs that control for the surgical approach are likely to provide useful insights into the aetiology and the development of therapies directed at postimplantation hearing loss. Key Words: Cochlear anatomy—Cochlear implant—Electrode insertion—Force—Imaging.

For the last 30 years, cochlear implantation (CI) surgery has been successfully used for the treatment of severe to profound deafness. With improvements in cochlear implant technology, the surgical criteria have expanded to include patients with residual low frequency hearing. These patients benefit from simultaneous use of the implant and a hearing aid in the same ear, termed electro-acoustic stimulation (EAS). EAS with preservation of residual hearing has been shown to be particularly effective for difficult perceptual tasks relying on temporal aspects of sound perception, such as listening in noise or to music (1,2). Despite best efforts, the potential for EAS is greatly diminished in approximately half of patients because of postimplantation hearing loss (3).

Postimplantation hearing loss may occur from direct and indirect damage to the cochlea (4). Direct damage arises from surgical access to the cochlea, either via cochleostomy or round window (RW) approach, and from electrode insertion trauma. Indirect damage to the cochlea is thought to occur from the biological response of the ear to surgery and can cause both postoperative and delayed losses of hearing. The early inflammatory reaction can lead to cell death of hair cells through processes such as apoptosis, necrosis, and necrosis-like cell death pathways (4). Delayed effects of the histopathological response include fibrotic change around the electrode, new bone growth, and a foreign body reaction (5,6), which may dampen cochlear mechanics through fixation of the round window (7), or lead to occlusion of the cochlear aqueduct (8) or ductus reuniens (9,10).

The precise contributions of direct and indirect cochlear damage to postimplantation hearing loss remain indeterminate, in part, because of the lack of a reproducible animal implantation model. The guinea pig cochlea...
is one of the most investigated animal models owing to an ease of access for implantation via cochleostomy (6) or RW approaches (11), a large enough size to accommodate a commercially available array (12) and the production of a histopathological response to the presence of an implant that is similar to other species, including humans (6). However, several studies investigating CI in guinea pigs have shown marked differences in trauma (6,13,14) and resultant tissue response, ranging from minimal (15–17) to almost total occlusion of the scala tympani of the lower basal turn (18–22). Similarly, postimplantation hearing loss has varied from substantial losses across all frequencies (17–19,21,23) to minimal hearing loss in selected frequencies (15,16,20,24–27) but with large interanimal variability. Electrode design and surgical factors are likely contributors to the variability so far observed; however, a less considered feature is the intricate anatomy of the guinea pig hook region (28,29) that may reduce its applicability for many modeling purposes. The aim of this study was to quantitatively describe important cochlear structures in the hook region and lower basal turn of the guinea pig for modeling inner ear surgery. The risks and variabilities of direct and indirect cochlear damage were assessed for a range of surgical approaches and electrode insertion profiles. Additionally, a theoretical method for determining the influence of surgical approach on the force of insertion at the first point of intracochlear contact was investigated.

METHODS

Thin-Sheet Laser Imaging Microscopy

All experimental procedures were approved by and performed in accordance with the Animal Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (ethics approval 14/299AU). Six tricolored guinea pigs were euthanized with sodium pentobarbitone (Lethabar; Virbac Australia Pty. Ltd., Milperra, NSW, Australia) administered intraperitoneally, and perfused transcardially with a 0.9% saline solution followed by 4% paraformaldehyde. Cochleae were removed and placed in 10% neutral buffered formalin before decalcification in 10% (w/v) EDTA. Cochleae were dehydrated by immersion in a graded ethanol series (50%, 70%, 90%, 100%, and 100% each for 30 min) to remove all hydration and facilitate deep penetration by the laser light (31). Spalteholz fluid (5:3 methyl salicylate: benzyl benzoate) was used as the clearing fluid. The TSLIM device consisted of a 400 mW green (532 nm) LASER illuminator (OEM 400 mW RGB Laser Chassis, OdicForce Lasers) mounted on a horizontal optical bench (Fig. 1). A 532 nm laser was selected as it stimulates fluorescence of a wide variety of biological structures (31). The beam was first passed through a Galilean beam-expander, then a beam-splitter creating two beam-lines (Fig. 1). The two beam lines were guided to opposite sides of a custom-designed specimen chamber, consisting of bonded microscope coverslips with an open top and filled with Spalteholz fluid. A cylindrical lens, an aperture, and ×10 microscope objective were used to focus each laser beam into a thin light sheet in a vertical plane. The LASER beams were positioned from opposite sides and orthogonal to the optical axis of a horizontally mounted Olympus MVX4 microscope (Olympus America, Inc., Center Valley, PA) that was attached to a Canon EOS 70D camera (Canon, Tokyo, Japan). Each cochlea was attached to a wooden rod that was placed in the middle of the specimen chamber and connected to three micropositioners (Thorlabs Inc., Newton, NJ), enabling x, y, z translations. Images were taken with the cochlea positioned at the intersection of the LASER sheets. Two column (y) and two row (x) images of optimal resolution were acquired at intervals of 10 μm in the z axis, totalling approximately 1,600 images per cochlea. Control of the micro-positioners was automated using a custom LabVIEW program (version 8.6; National Instruments, Austin, TX) and image stitching of the four cross-sectional images into a composite image (Fig. 2A) was automated with MatLab (version 15b, Mathworks Inc., Natick, MA).

Segmentation and Reconstruction

The z stack of stitched images was imported into Amira 5.4 (Visualization Sciences Group, France) for image analysis. The segmentation and reconstruction of the fluid spaces and anatomical structures using Amira’s segmentation tool have been previously documented (28). Specific spaces and structures were defined and selected (Fig. 2B). In both humans and guinea pigs, all capillary regions empty into the principal vein of the cochlea, termed the vein of the cochlear aqueduct or the inferior cochlear vein (32). An interspecies difference is that the human cochlea has two spiral veins and several other important venous routes that empty to the principal vein (32). Conversely, the guinea pig cochlea has a single spiral vein that drains almost the
entire cochlea, excluding a small region of the most basal end that is drained by the vein of the round window and the posterior vestibular vein. All three veins were thus labeled (Fig. 2B). The cochlea was oriented in anatomical position using the otic capsule as a reference point and with the adjacent RW directed caudally and dorsally (33). Three RW approaches (midpoint, inferior, and anteroinferior margins), visible from the surgeon’s perspective after opening the dorsal bulla (6), were used to measure each point’s proximity to several important intracochlear structures (28). The shortest distance of these intracochlear structures to eight cochleostomies distributed across the surface of the otic capsule from the surgeon’s perspective was also determined. Cochleostomies were in a predetermined plane (inferior, anteroinferior, or anterior) and distance from the mid-point of the RW (0.5, 1.0, or 1.5 mm) (Fig. 3). Since the diameter of a drill burr for cochleostomy formation is commonly 1.0 mm (34), intracochlear structures were deemed to be at direct risk from the surgical approach if they were located less than 0.5 mm away. The cochlear hook region was defined as structures near to, and including the most basal portion of the cochlear duct (35).

Modeling the Ideal Insertion Vector

The ideal insertion vector for straight electrodes is in line with the longitudinal (or centerline) axis along the lower basal turn of scala tympani (36,37). For determining this ideal vector, a spline of the centerline of scala tympani was created. This was achieved by determining the intersection of the longest horizontal and vertical axes of scala tympani cross-sections every 5 degrees from the round window to the end of the lower basal turn. A second spline, representing the ideal insertion vector, was placed from each surgical approach (cochleostomy and RW) to the furthest point along the centerline spline while maintaining a margin of at least 0.2 mm from all intracochlear structures. 0.2 mm was chosen as it is the radius of the tip of several contemporary clinical (28) and animal electrode arrays (6,12). The ideal insertion vector was then extended from the centerline spline to the outer wall of scala tympani, which is deemed a “less traumatic” point of first contact (38). The entire tracing was converted into Cartesian coordinates.

Since the outer wall of the scala tympani is a three-dimensional curve, two views (axial and radial) were used for investigating the angular components of the ideal insertion vector upon contact with the outer wall (39). A stepwise flowchart is shown in Figure 4. First, coordinates of a $5 \times 5$
grid, approximately 25 \( \mu m^2 \) in size, were collected around the point of contact using Amira’s spline tool. MatLab was used to generate a spherical surface to the grid coordinates using the least squares technique. A “junction point” between the insertion vector and the spherical surface was determined and a tangent plane to this junction point was created. The axial plane, closely corresponding to the Cochlear View in clinical use (40), was defined as the plane normal to the modiolus and passing through the junction point.

The line, formed by the intersection of the tangent and axial planes (the “intersection line”), enabled the analysis of an angle (\( \alpha \)) between the projections of the tangent line and an ideal insertion vector on the axial plane (Fig. 5A). TSLIM has since been performed on implanted cochlea in our laboratories (under review) and an example of the axial plane with \( \alpha \) angle is shown in Figure 5A. The force exerted on the outer cochlear wall rises with the \( \alpha \) angle, such that as \( \alpha \) approaches 90°, all force applied by the surgeon in the line of electrode insertion would be transmitted onto the outer wall without any advancement (41). Conversely, as \( \alpha \) approaches 0 degree, all forces applied by the surgeon in the line of insertion would contribute to advancing the electrode and with no force exerted onto the outer wall (40).

The radial plane was defined as normal to the intersection line and passing through the junction point. This radial plane corresponds approximately to a cross-section of the basal turn (39). An angle (\( \beta \)) was then determined between the projection of the ideal insertion vector and the tangent line on the radial plane (Fig. 5B). The \( \beta \) angle is inversely related to the upward force, in the radial or \( z \) direction. For example, as \( \beta \) approaches 0 degree, all forces applied by a surgeon in the line of insertion would be directed toward the under-surface of the basilar membrane and osseous spiral lamina (OSL), with a greater likelihood of penetrating scala vestibuli (41). Conversely, as \( \beta \) approaches 90 degrees, minimal radial force would be applied.

For determining the angular insertion depth of the point of first contact with the outer wall, the midpoint of the RW was used as the zero reference angle (42). MatLab was then used to generate two planes, both passing through the modiolus but with different origins at the midpoint of the RW and point of first contact with the outer wall. Next, two intersecting vectors were formed from each plane as it intersected with the axial plane, thus providing an angular depth of insertion around the modiolus (termed \( \delta \) in Fig. 5A). A linear distance from the point of first contact to the midpoint of the RW was also determined using Amira’s three-dimensional measurement tool.

**RESULTS**

**Surgical Approach**

Figure 2B shows a reconstructed and segmented guinea pig cochlea. The venous system was most closely related to the inferior margin of the RW and adjacent otic capsule (Fig. 6A). Inferior cochleostomies 0.5 to 1.0 mm from the RW were located on average less than 0.5 mm from the cochlear venous system (Table 1). The OSL, basilar membrane, spiral ligament, scala media, and scala vestibuli followed a course from the posteroinferior margin of the RW to the anterior otic capsule. A RW approach via the anteroinferior margin provided the safest distance from these cochlear structures, all with a mean distance of >0.50 mm (Fig. 6A). Anterior cochleostomies, when controlling for distance from the RW, were at greatest risk of direct damage to intracochlear structures, excluding the cochlear venous system (Table 1). Cochleostomies placed further from the RW were closer to most intracochlear structures, which is likely a result of a more narrowed cross-sectional area of the scala tympani as it spirals towards the cochlear apex.

**Ideal Insertion Vector**

The angular depths of insertion from cochleostomies to their point of first contact are shown in Figure 7. Median linear distance for these cochleostomies ranged from 3.0 to 4.1 mm. Cochleostomies placed further from the RW and in either an inferior or anteroinferior plane achieved the deepest insertions, using either linear or angular measurements (Fig. 7). Overall, cochleostomies had considerable interindividual variability for their insertion depths, with a range of greater than 50 degrees for some locations. RW approaches achieving deepest angular insertion were via the anteroinferior margin (median of 97.5 degrees), followed by midpoint (93.2 degrees), and inferior margin (86.6 degrees).

Insertion vectors using a RW approach from either the midpoint or inferior margin exerted higher outer wall (i.e., large \( \alpha \) angles) and radial forces (i.e., small \( \beta \)
angles) compared with the anteroinferior margin (Fig. 8). An inferior cochleostomy placed 1.5 mm from the RW exerted the greatest outer wall force, with a mean \( \alpha \) angle of 74.6 degrees (Fig. 9). An anterior cochleostomy placed 1.0 mm from the RW exerted the least outer wall force (mean \( \alpha = 41.5 \) degrees) but greatest radial force with a mean \( \beta \) angle of 66.0 degrees. Most cochleostomies had large interindividual variability for \( \alpha \) angles, conversely

![Image](image_url)

**FIG. 5.** A, TSLIM axial plane image of an archival implanted left guinea pig cochlea showing the tissue response to implantation. At the bottom of the picture is the cochleostomy in proximity to the round window. “\( \alpha \)” lies between the two solid arrows representing the insertion vector and the tangent line in this plane. Two dotted arrows represent vectors of the midpoint of the RW to the modiolus and from the modiolus to the point of first contact on the lateral wall. Between them is the angular depth of insertion “\( \theta \)”. B, Radial plane of an unimplanted left cochlea showing “\( \beta \)” angle between the insertion vector, as it traverses scala tympani, and the tangent line. TSLIM indicates thin-sheet laser imaging microscopy.

**FIG. 6.** Shortest distance between cochlear structures and through either a cochleostomy (CO) or round window (RW) approach. A, Mean guinea pig results (n = 6) from the present study. CO positions were 1.0 mm from the mid-point of the RW. B, Replotted results from Li et al. (2007) of a single human temporal (28). Al indicates anteroinferior plane; Ant, anterior plane; Inf, inferior plane; Mid, mid-point.

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angles were relatively stable irrespective of the plane of the cochleostomy or distance from the RW (Fig. 9).

Location of the Cochlear Aqueduct and Ductus Reuniens

The ductus reuniens was most closely related to the inferior margin of the RW (Fig. 6A), in which the distance was on average less than 1 mm. All cochleostomies were located more than 1 mm from the ductus reuniens. The cochlear aqueduct traversed near the inferior margin of the RW and adjacent otic capsule (Fig. 6A), whereupon it was at considerable risk from proximal inferior cochleostomies (mean distance <0.5 mm).

DISCUSSION

Safety of the Anteroinferior Margin of the RW and Adjacent Otic Capsule

The anteroinferior margin of the RW or adjacent otic capsule of the guinea pig allowed direct implantation into scala tympani, with a safe margin from important cochlear structures. Anterior cochleostomies were more likely to result in drilling or electrode insertion trauma to the basilar membrane or spiral ligament, and even direct entry into scala media. Cochleostomies placed inferiorly had the greatest risk of direct venous damage. Of note, the anatomical results of this study gave no evidence that an accurately placed anteroinferior cochleostomy is more favorable than via an extended anteroinferior RW approach.

The Risk of Injury to the Ductus Reuniens and Cochlear Aqueduct

The cochlear aqueduct and ductus reuniens in the guinea pig were found to be in proximity to the inferior margin of the RW. While the risk of direct damage to these structures was low for most surgical approaches (>0.50 mm in distance), indirect damage could potentially result from extension of a robust tissue response. Occlusion of the ductus reuniens from trauma or tissue response is one proposed mechanism for the

### TABLE 1. Shortest distance between cochlear structures and different cochleostomy locations defined in plane (anterior, inferior, or anteroinferior) and distance from the RW

<table>
<thead>
<tr>
<th>Plane of Cochleostomy</th>
<th>Distance From RW (mm)</th>
<th>Scala Media</th>
<th>Scala Vestibuli</th>
<th>Basilar Membrane</th>
<th>Spiral Ligament</th>
<th>Osseous Spiral Lamina</th>
<th>Venous System</th>
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</thead>
<tbody>
<tr>
<td>Inferior</td>
<td>0.5</td>
<td>1.08 (0.82–1.20)</td>
<td>1.17 (1.01–1.29)</td>
<td>1.08 (0.77–1.31)</td>
<td>1.04 (0.73–1.20)</td>
<td>1.01 (0.79–1.10)</td>
<td>0.42 (0.11–0.87)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.04 (0.90–1.11)</td>
<td>1.14 (1.06–1.20)</td>
<td>0.99 (0.85–1.09)</td>
<td>1.00 (0.86–1.10)</td>
<td>1.01 (0.90–1.09)</td>
<td>0.39 (0.12–0.66)</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.75 (0.63–0.82)</td>
<td>0.88 (0.73–1.11)</td>
<td>0.70 (0.57–0.78)</td>
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<td>0.76 (0.69–0.87)</td>
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</tr>
<tr>
<td>Antinf</td>
<td>0.5</td>
<td>0.91 (0.81–1.02)</td>
<td>1.09 (0.96–1.20)</td>
<td>0.88 (0.77–1.02)</td>
<td>0.86 (0.73–1.01)</td>
<td>0.92 (0.75–1.06)</td>
<td>0.72 (0.53–1.08)</td>
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<td></td>
<td>1.0</td>
<td>0.80 (0.72–0.90)</td>
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<td>0.77 (0.67–0.89)</td>
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<tr>
<td></td>
<td>1.5</td>
<td>0.50 (0.40–0.62)</td>
<td>0.74 (0.66–0.92)</td>
<td>0.47 (0.37–0.62)</td>
<td>0.45 (0.36–0.61)</td>
<td>0.64 (0.57–0.86)</td>
<td>0.97 (0.70–1.25)</td>
</tr>
<tr>
<td>Anterior</td>
<td>0.5</td>
<td>0.70 (0.56–0.85)</td>
<td>0.88 (0.77–1.04)</td>
<td>0.67 (0.56–0.81)</td>
<td>0.64 (0.52–0.76)</td>
<td>0.76 (0.58–0.87)</td>
<td>0.92 (0.61–1.26)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.48 (0.37–0.55)</td>
<td>0.73 (0.51–0.84)</td>
<td>0.47 (0.34–0.56)</td>
<td>0.33 (0.21–0.42)</td>
<td>0.60 (0.56–0.65)</td>
<td>1.97 (1.01–1.37)</td>
</tr>
</tbody>
</table>

Mean and range (minimum and maximum values) are shown.

RW indicates round window.
development of endolymphatic hydrops that has been observed in implant recipients (8–10), so caution must be exercised when the electrode insertion is placed inferiorly to the RW. Cochlear aqueduct occlusion from extensive tissue response has similarly been noted in the temporal bone of an implant recipient with delayed hearing loss (8). However, the precise role of the cochlear aqueduct and the effect of occlusion on hearing remain uncertain (43).

### Ideal Insertion Vector

Inferior cochleostomies placed farthest from the RW were found to achieve the deepest insertion depth (mean of 146.2 degrees) but with the lowest likelihood of advancement due to high outer wall forces. This result likely arises from a deeper insertion contacting the outer wall at a point where there is greater curvature of the basal turn of the cochlea. Conversely, a shallow insertion arising from a more proximally located cochleostomy contacts the outer wall where there is a gentler curvature of the cochlea, thus causing a lower α angle and initial outer wall force. Another consideration is the accumulative force of the insertion, as opposed to the instantaneous force of the first contact. For example, a shallow insertion onto the outer wall may have longer travel along the outer wall than a deep insertion, resulting in higher overall frictional forces that must be overcome to advance the electrode. Hence, a shallow insertion may have a lower initial outer wall force compared with deep insertion, based upon present modeling, but potentially a similar or greater accumulative force as it travels along the outer wall. Angular descriptions instead of force measurements were presented in this study because force results are dependent on electrode features, such as the behavior of the tip, and the friction coefficient of the outer wall surface (39).

The structural relationships in this study are consistent with human three-dimensional anatomical studies (28,29,35), however, with approximately half the magnitude owing to the smaller dimensions of the guinea pig hook region and lower basal turn (Fig. 6). The safety of an anteroinferior placed cochleostomy concurs with results of human anatomical studies (28,44) and implantation studies with ex vivo temporal bones (45,46). Subtle variations in cochleostomy placement or round window entry may also explain the unpredictability in angular depths (41,47,48) and trauma (47,49) identified among implant recipients. However, this inference from current findings is cautioned by tighter coiling of the guinea pig cochlea that results in a much more rapid transition from the hook to the spiral region. For example, the angular depth to point of first contact in human modeling ranges from 110 to 180 degrees (48,50), which is a similar range to cochleostomies in the present study. However, this range of angular depths translates to a linear distance of 8 to 12 mm in humans compared with 3 to 4 mm for ideal insertion vectors in the guinea pig. Hence, small differences, perhaps less than 0.10 mm, in the placement of the cochleostomy or of the round window entry result in a much larger discrepancy when extrapolated onto the tightly coiled outer wall of the guinea pig cochlea. Accurate placement of the cochleostomy or RW entry is therefore paramount in experiments where a consistent level of trauma is required, such as studies investigating treatment effects on the histopathological response. Furthermore, a correct trajectory of the electrode is particularly crucial during the early stages of implanting in guinea pigs because of the relatively short distance to the outer cochlear wall.

Angular measurements in axial and radial planes have been presented as they are more translatable to the surgeon’s and histopathologist’s views. The radial plane directly relates to important neural and soft tissue structures but is poorly defined with current imaging techniques of implant candidates. Modeling was performed exclusively with straight insertion vectors since only straight electrodes can be reliably used for both RW and cochleostomy approaches (51). Presented methods for the angular descriptions of the ideal insertion vector could be applied to human cochleae. Crucially, the ideal insertion vector has not been analyzed experimentally for the preservation of hearing or structural integrity.

In summary, careful cochleostomy placement is essential for minimizing cochlear trauma and for ensuring that a straight vector successfully advances on the outer wall with low radial force and risk of dislocation of the cochlear partition. Subtle variations in the surgical approach and insertion vector may partly explain the unpredictability of the angular depth of insertion, surgical trauma, and hearing outcomes among experimental studies. The guinea pig hook region and lower basal turn have similar structural relationships to humans. Therefore, experiments with guinea pigs that control for the surgical approach are likely to provide useful insights into the aetiology and the development of therapies directed at postimplantation hearing loss.
Acknowledgments: The authors thank Professor Joseph Nadol for permitting the replotting of data in Figure 6B.

REFERENCES


3.3 Additional Discussion

There are some limitations in the present study that warrant further discussion. Firstly, other important factors that can dictate the path of insertion were not accounted for in the present study. These include middle ear anatomy (removed for tissue processing in the present study), common anatomical variants (of the inner or middle ear), along with the surgeon’s view and surgical exposure for performing the insertion. For example, a round window niche overhang or an obstructed view through the transfacial recess can often dictate the approach in humans, such as deciding on a more accessible cochleostomy location, reverting to a round window approach or even the initial trajectory of the implant (Iseli et al., 2014). Secondly, cochlear implantation surgery in humans can be performed from several approaches (including posterior tympanotomy, suprameatal, endural), all with differing ideal electrode trajectories to consider (Mangus et al., 2012). Conversely, in guinea pigs, a postauricular approach is standard (Connolly et al., 2011, Lee et al., 2013, James et al., 2008, Rah et al., 2016, Rowe et al., 2016). With these limitations in mind, extrapolating the findings of the present study to humans should be done with considerable caution.

Damage to the endosteum during cochleostomy, perhaps from vigorous drilling or the entry of the implant, can cause a fibrotic reaction that can extend in a retrograde direction onto the round window membrane (Quesnel et al., 2015). This has been previously reported with a contemporary electrode in an implant recipient who later experienced delayed hearing loss (Quesnel et al., 2015), perhaps from increased cochlear impedance (Nageris et al., 2012). The prevalence and extent of this fibrotic reaction is not yet known and so suggesting a ‘safe’ distance of cochleostomy placement for reducing this reaction is not possible. Implanting the electrode directly through the round window membrane itself would negate immediate risk of endosteal damage, however, this approach has still resulted in greater low frequency hearing loss when compared to carefully placed cochleostomies in a similar guinea pig model (Rowe et al., 2016). As such, a safe distance for cochleostomy placement to reduce the fibrotic reaction along with other methods to reduce endosteal damage, such as surgical robotic drills, warrant further investigation.
Chapter 4: Efficacy of Preoperative Steroids in Cochlear Implantation Surgery
Chapter 4: Efficacy of Preoperative Steroids in Cochlear Implantation Surgery

4.1 Introduction

Among a few small clinical studies, glucocorticosteroids (or ‘steroids’) have shown some promise for preserving hearing and reducing electrode impedance (Paasche et al., 2009, Enticott et al., 2011, Rajan et al., 2012, Sweeney et al., 2015, Carlson et al., 2011, Kuthubutheen et al., 2012, Kuthubutheen et al., 2017). In addition, animal studies have provided further insight in the treatment efficacy of steroids. Systemically delivered steroids, usually via intravenous means, have shown the most promise for hearing preservation, but with considerable variation in outcomes. Results for post-implantation hearing loss have included generalised (Quesnel et al., 2011, Lee et al., 2013), incomplete or transient (Connolly et al., 2011, Rah et al., 2016, Kuthubutheen et al., 2015) improvements compared to control animals. Reductions in the tissue response have been widely identified, although in the presence of highly variable levels of trauma, ranging from minimal (Kuthubutheen et al., 2015, Rah et al., 2016, Connolly et al., 2011) to significant trauma (Lee et al., 2013). And one proposed hypothesis for the inconsistent results of steroid protection is a variance in the level of trauma. Glucocorticosteroids have a broad range of anti-inflammatory and immunosuppressive properties (Bas et al., 2012) but their actions vary depending on the cell population, their physiological properties and the inciting injury (Boumpas et al., 1993). Therefore, the aim of this study was to determine if graded trauma could alter the level of hearing protection and inhibition of fibrosis afforded by systemic steroids.

An in vivo study with guinea pigs was designed to address this aim using the least traumatic technique of an anteroinferior cochleostomy placed close to the RWM (from the findings of Chapter 3). A graded, and site-specific, level of trauma was attempted by implanting to three designated depths (1.5, 3.0 and 5.0 mm). While shortened electrodes were initially used for EAS patients to preserve the structural integrity and residual hearing, this has not been conclusive shown in clinical studies (Santa Maria et al., 2014, Causon et al., 2015) nor in experimental animal models. Finally, the surgeons attempt at a controlled cochleostomy position, relative to the RWM, was analysed for variability and relationships to the level of
trauma, hearing loss and tissue response.

Morphological, whole-specimen analysis was again performed using the TSLIM protocol developed in Chapter 3. It was anticipated that TSLIM would enable a continuous visualisation of the electrode path and tissue response compared with the images that are produced by much thicker histological sectioning. A compromise of TSLIM is the inability to distinguish the different components of the tissue response, such as osteoneogenesis and dense fibrosis.

The specific hypotheses tested in this study were that:

i) Deeper inserted electrodes (with cochleostomy position controlled for) cause greater site-specific levels of trauma

ii) Deeper inserted electrodes cause greater low frequency hearing loss

iii) Systemic steroids reduce post-implantation hearing loss and tissue response in the presence of minimal trauma only

iv) Placement of the guinea pig cochleostomy, defined as distance from the RWM, can be achieved with high repeatability

4.2 Otology & Neurotology Publication
The Role of Preoperative Steroids in Atraumatic Cochlear Implantation Surgery


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**Hypothesis:** Depth of insertion is related to the extent of tissue response and low frequency hearing loss. Intravenous steroids have greatest effect in reducing postimplantation fibrosis and hearing loss in the presence of significant electrode insertion trauma, when compared with saline treatment.

**Background:** Experiments exploring the enhancement of cochlear implantation (CI) outcomes with glucocorticosteroids have produced mixed results, possibly due to lack of standardization of the CI model.

**Methods:** Forty-eight normal-hearing guinea pigs were randomly implanted with a highly flexible electrode to a depth of 1.5, 3.0, or 5.0 mm. For each insertion depth, sub-cohorts received either intravenous saline ("saline") or dexamethasone ("steroid") 60 minutes before implantation. Shifts in electrocochleography thresholds at 2 to 32 kHz were determined before and 4 weeks after implantation. Cochleae were harvested and imaged.

The preservation of residual hearing has become a recognized goal of cochlear implantation (CI) surgery to optimize the performance of perceptual tasks relying on pitch resolution, such as listening in noise or to music (1,2). Despite considerable improvements in electrode design and surgical technique, complete hearing preservation to within 10 dB of preoperative hearing occurs in only a third of patients (3). In recent years, glucocorticosteroids have attracted attention as a potential pharmacological intervention for hearing preservation CI surgery. So far, steroids have been administered systemically, locally, or via elution from a cochlear implant. Among a few small and low-powered clinical studies, steroids have shown some promise for preserving hearing and reducing electrode impedance (4–9). Animal studies have provided further insight in the treatment efficacy of steroids, although considerable variation in outcomes exists. For example, hearing protection has varied from generalized (10,11), incomplete or transient (12–17), to no protection (18,19). Among these studies, the extent of intracochlear scarring and foreign body reaction has also varied from minimal (18) to near complete obliteration of the scalae that may (10) or may not be (12,16,19) associated with significant surgical trauma.

With such marked differences between hearing loss, trauma, and the level of protection afforded by steroids, it has been difficult to understand how steroids act to reduce hearing loss, and more importantly, whether the circumstances in which they do could be expected to apply to humans. The purpose of this study was to reconcile some of these inconsistencies by exploring the relations between site-specific cochlear trauma and steroid administration. A secondary outcome was steroid effect on hearing loss. Systemic steroids were examined because of their widespread use in clinical practice, and from the outcomes of previous studies that have shown a reduction in tissue response and postimplantation hearing loss in experimental CI (10,12,16). Morphological
analysis was performed using a high resolution, whole specimen imaging technique, which enabled a detailed continuous assessment of cochlear anatomy and pathology.

MATERIALS AND METHODS

Animals and Experimental Design
All procedures in this study were approved by and performed in accordance with the Animal Ethics Committee of the Royal Victorian Eye and Ear Hospital (Project 14.299AU). Forty-eight adult Dunkin-Hartley tricolour guinea pigs, weighing more than 400 g, were used in this study. Animals underwent left sided implantation using a flexible electrode to a randomly assigned depth of 1.5, 3.0, or 5.0 mm. This approach was used to control the extent of cochlear trauma. Deeper insertions typically cause greater trauma (20) since they are placed further in towards the apex of the cochlea, where there is a reduced cross-sectional area and a greater radius curvature (21). For each insertion depth, sub-cohorts received either intravenous dexamethasone (‘steroid’) at 2 mg/kg or saline (‘control’) 60 minutes before implantation. Electrocochleography (ECochG) recordings were performed before surgery and at the survival end-point of 4-weeks. Cochleae from all animals were removed and cleared to transparency, after which they were serially imaged using thin-sheet laser imaging microscopy (TSLIM). The researcher was blinded to the treatment groups for hearing and TSLIM analyses.

Cochlear Implant and Recording Electrodes
The cochlear implant was a multichannel electrode array that was 15 mm in length and with three platinum rings at its tip, welded 0.75 mm apart. Each platinum ring was connected to an internal platinum wire that was 25 μm in diameter and tightly coiled as a helix to resemble contemporary human electrode designs (22). All parts were embedded in a silastic carrier used in current prostheses (MDX4–4210 Dow Corning Products, USA). The maximum diameter of the implant was 0.45 mm, narrowing to 0.41 mm towards the tip. A small silastic plug was placed at one of three distances (1.5, 3.0, or 5.0 mm) from the tip of the electrode thereby designating the depth of insertion. ECochG was recorded with a 0.5 mm diameter gold ball electrode placed directly on the round window membrane (RWM) and a subcutaneous needle electrode placed on the nape. At the end of the survival period, the wound was reopened (RWM) and a subcutaneous needle electrode placed on the

Acoustic Stimuli
Acoustic stimuli were delivered via a loudspeaker (Richard Allen DT-20, Portsmouth, UK) placed 0.1 m from the test ear. The contralateral ear was occluded using an ear mould compound (Otoform K2, DLT, West Yorkshire). Using this method, the frequency-specific cross-head attenuation (measured from the occluded ear canal of the guinea pig) is 28, 27, 35, 45, and 28 dB at 2, 4, 8, 16, and 32 kHz, respectively (23). The stimuli for ECochG recordings were computer generated tone pips of 20 ms duration with 1 ms linear onset/offset ramps, presented at 2, 4, 8, 16, 24, and 32 kHz. These were presented every 50 ms and averaged over 25 repetitions.

Recording Configurations
Responses were preamplified by a factor of 1.000 (DAM-5A, W-P Instruments Inc., New Haven, CT) and then band-pass filtered (Krohn-Hite 3750) at 0.1Hz to 100,000kHz. The

For ECochG recordings were computer generated tone pips of 20 ms duration with 1 ms linear onset/offset ramps, presented at 2, 4, 8, 16, 24, and 32 kHz. These were presented every 50 ms and averaged over 25 repetitions.

Responses were digitized by a 24-bit analogue-to-digital converter (DT9847S) that sampled at 216 kHz. Alternating stimuli with condensation and rarefaction phases were saved separately, allowing them to be added to derive a compound action potential (CAP). CAP traces were then digitally filtered between 10 and 1500 Hz. Hearing thresholds were visually inspected in 5 dB decrements and determined as corresponding to the minimal intensity for which a CAP was clearly recognized (11). The maximum output plus 5 dB was recorded if no responses were present to the maximum output (24). An illustrative dataset of CAP responses to an 8 kHz tone is shown in Figure 1.

Surgery
All animals received a standardized drug administration and implantation procedure. High-dose systemic dexamethasone was administered into the right jugular vein before the commencement of the implant surgery (10,12). Anesthesia for surgical procedures and ECochG recordings was inducted and maintained with intramuscular ketamine (60 mg/kg, Troy Laboratories Pty, Ltd, Sydney, Australia) and xylazine (4 mg/kg, Troy Laboratories Pty Ltd). The local anesthetic lignocaine (1 mg/mL, Troy Laboratories Pty Ltd) was injected before a postauricular incision. The left cochlea was then approached after tissue dissection and use of a 3 mm cutting burr to create a bullostomy. A 0.8 mm diamond burr was used to create a cochleostomy approximately one width of the drill bit from the RWM in an antero-inferior plane to avoid important intracochlear structures. This location has recently been validated by modelling guinea pig cochleae in our laboratories (under review). Flushing away of visible bone dust was performed and direct suctioning of perilymph was avoided. The cochlear implant was passed at a constant slow speed (approximately 10 mm/min) in to the cochlea to the depth designated by the silastic plug or until first resistance was met. The implant was secured proximally to the edge of the bullostomy with carboxylate cement (DurelonTM, 3M ESPE AG, Seefeld, Germany), while avoiding the entry of cement in to the middle ear.

Thin-sheet Laser Imaging Microscopy
After transcerebral perfusion with phosphate-buffered normal saline, cochleae were removed and placed in 10% neutral buffered formalin before decalcification in 10% (w/v) ethylenediaminetetra-acetic acid for 3 weeks. Cochleae were dehydrated by immersion in a graded ethanol series (50, 70, 90, 90, 100, and 100% each for 30 mins) followed by immersion in hexane. TSLIM optically sections tissue using a thin sheet of light that induces an orthogonal plane of auto-fluorescence. Since the guinea pig cochlea is opaque and surrounded by bone, each specimen was cleared to transparency with Spalteholz fluid (5:3 methyl salicylate: benzyl benzoate) to avoid light scatter in the tissue and achieve a deeply penetrating image (25). Staining or fluorescent techniques were avoided because of possible distortion of the intracochlear anatomy. As such, meaningful analyses of cellular and fibrinous tissue responses (26), and neurosensory cell counts were not possible.

The TSLIM device consisted of a 400 mW green (λ = 532 nm) laser illuminator (OEM 400 mW RGB Laser Chassis, OdicForce Lasers, UK) mounted on a horizontal optical bench rail. Details of the Laser and camera configuration have been previously described (25). In summary, two Laser beams were focused on to the cochlea specimen from opposite sides. Images were obtained via an Olympus MVX4 microscope (Olympus America, Inc., Center Valley, PA) attached to a
Canon EOS 70D camera (Canon, Tokyo, Japan) placed orthogonal to the Laser beams. Image stitching of four cross-sectional images into a composite image was automated with MatLab (version 15b, Mathworks Inc., Natick, MA).

Detailed visual inspection of the lower basal turn of the cochlea was performed using Amira 5.4 (Visualization Sciences Group, France). The implant’s position was inferred from its impression on the tissue reaction, typified by a circumferential or semilunar space in the tissue. To estimate the extent and location of tissue response to the electrode, relevant voxels were labeled using Amira’s Segmentation Editor and were selected using the paintbrush on every 3rd slice. The limits of the scala tympani were similarly labeled. The center of the RWM was determined as a zero-reference angle since it is in close approximation to the most basal region of the organ of Corti (27).

Tissue response and the path of the electrode were then determined for every 30 degrees from the zero-reference angle in a plane of rotation with the modiolus at its center. Ossicular spiral lamina (OSL) fracture was defined as a break in the internal lamina, with or without the presence of osteoneogenesis.

Finally, a cochleotopic map was created by passing a spline along the tunnel of corti, mapping length along this line to Wada’s equations (28), adapted from Greenwood's guinea pig work (29).

**Statistical Analyses**

Statistical analyses were performed with IBM SPSS version 23.0 (IBM Corp., Armonk, NY). For parametric data, analysis of variance (ANOVA) was used. When repeated-measures were analyzed, ANOVA was applied with post-hoc testing using a Bonferroni correction for multiple comparisons. Unless otherwise stated, all residuals were suitably normal and had constant variance.

**RESULTS**

**Electrode Position Within the Cochlea**

The mean angular depth of insertion for shallow, mid-depth, and deep insertions were 53.0 degrees (±6.7 standard error of the mean [SEM]), 84.9 degrees (±11.0), and 144.4 degrees (±10.3). The corresponding cochleotopic places were approximately 24 to 16, 16 to 12, and 12 to 8 kHz, respectively. The mean location of the cochleostomy was 27.4 degrees (±1.43 degrees SEM) of rotation from the midpoint of the RWM.

**Global Tissue Response**

The total volume of the tissue response within the cochlea was greater in the controls than any of the steroid groups (Fig. 2). Univariate ANOVA analysis revealed a significant effect with intravenous treatment (p = 0.032) and depth of insertion (p = 0.012). No significant interaction was found between these factors. Post-hoc testing using the Bonferroni correction revealed a significant difference between deep and shallow (p = 0.01) insertions only.

**Regional Tissue Response**

To better explore the intracochlear pathology, the relations between fibrosis and cochlear place were explored. This was achieved by calculating the cross-sectional area of the tissue response within 30 degrees cochlear segments beginning at the RWM (Fig. 3). These data were nonparametric and therefore normalized by a logarithmic transformation before being subjected to statistical analysis. A significant effect of intravenous treatment upon fibrosis (p = 0.04) was identified on a repeated measures ANOVA and with degrees of rotation from the round window as a within subject variable. Because of the effect of cochlear place on the tissue response, subsequent analyses were reported by cochlear region.

The tissue response at 30 degrees was found to be in continuity with the cochleostomy for all animals. The mean occlusion of scala tympani in this region was approximately 0.2 for all experimental groups (Fig. 3). Eight animals had a retrograde extension of the tissue response from the cochleostomy to the RWM that involved more than 5% of its endothelial lining (Fig. 4A). This pathology was seen in electrodes of all insertion depths. Four of these eight animals had a concurrent hook
region fracture, while the other four animals had early OSL contact (30–90 degrees). One other animal undergoing deep implantation had a distal fracture of the OSL (120–150 degrees) that was associated with a profuse tissue response (proportion of 0.86 occupying scala tympani) but without involvement of the RWM.

The region with greatest tissue response for both deep and mid-depth insertions was 60 to 90 degrees from the RWM. Contact with the endosteum at either the modiolus or OSL most frequently occurred in this region (Fig. 4C). Lateral wall contact was most frequently made by deep electrodes at 120 degrees from the round window. After initial contact, these electrodes remained in proximity to the lateral wall and usually abutted the basilar membrane but without elevating it (Fig. 4D).

Apical extension of the tissue response of at least 30 degrees (or approximately 1 mm) beyond the tip of the electrode occurred in five animals undergoing deep and mid-depth insertions. One of these animals had a hook region fracture. The tips of these electrodes contacted the OSL (two animals) and lateral wall (3), with all electrode paths avoiding contact to the basilar membrane.

Effects of Steroids on Fibrosis by Cochlear Region

For shallow and mid-depth insertions, steroids had no effect on the area of fibrosis up to 60 degrees, namely the region of the RWM, cochleostomy, and hook (Fig. 3). Most of the animals with significant cochlear trauma or fibrosis in this region had, per chance, been treated with steroids (all five animals with OSL fracture were preadministered steroid and six of eight animals with fibrotic involvement of the RWM). Since surgical trauma was not balanced between the saline- and steroid-treated cohorts, a sub-analysis was performed of the tissue response at 30 to 60 degrees and excluding animals with cochlear trauma. The mean occlusion of scala tympani in the 30 to 60 degrees regions of remaining animals was less than 0.3 for all experimental groups. On repeated measure ANOVA (with degrees of rotation as a within subject variable), a significant effect of steroid treatment on tissue response was identified \((p = 0.02)\). No significant interaction was found between these factors.

In deeply inserted electrodes, there was a significant reduction \((p = 0.03)\) in fibrosis following steroid treatment at 90 to 150 degrees (Fig. 3) on repeated measure ANOVA. This was the region of the cochlea where the electrode contacted the basilar membrane and lateral wall (Fig. 4D).

Electrophysiology

On a repeated measures ANOVA, with frequency as a within subject variable, preoperative hearing did no
differ between intravenous treatments, depth of insertion, nor with any two- or three-way interaction. CAP threshold shifts at 4 weeks were similar in magnitude between intravenous treatments, but differed subtly with insertion depth (Fig. 5). Mean losses were less than 20 dB across all experimental manipulations. Shallow insertion caused a threshold shift across the high frequency range of 16 to 32 kHz (Fig. 4) while thresholds in response to lower frequency stimuli (2–8 kHz) remained unchanged. Mid-depth and deep insertions in control animals caused greater than 5 dB threshold shifts for all frequencies above 2 kHz. For deep insertions, the mean threshold shift at 2 kHz was greater than 5 dB.

Regional Trauma and Hearing Loss

In the eight animals where fibrosis had extended retrograde from the cochleostomy to involve the RWM, low frequency hearing (2- and 4-kHz) was poorer than expected, averaging 15.6 dB (±3.4 dB SEM). All animals with a hook region fracture had both a robust tissue response and considerable high frequency hearing loss (28.5 dB, ±4.2 dB SEM averaged across 24- and 32-kHz).

DISCUSSION

Overview of Results

Intracochlear trauma and fibrosis were found most frequently near the cochleostomy and where the electrode negotiated the hook region. Systemic steroids reduced the volume of fibrosis with deeper insertions and in the more apical regions of the cochlea, where contact between the electrode and basilar membrane frequently occurred. Steroids reduced fibrosis around the cochleostomy, but only in the absence of severe cochlear trauma. Fracture of the OSL and/or fibrotic involvement of the RWM exacerbated hearing loss.

Insertion Depth and Trauma

In most animals, implanting a highly flexible electrode caused no observable macroscopic trauma. Although infrequent in occurrence, OSL fracture could be found with all depths of insertion and was largely confined to the region of the hook. A poor initial trajectory is likely to be the cause of trauma in this setting (30). Physical contact (with potential trauma at the microscopic level) of the basilar membrane that has been demonstrated in

![FIG. 4. A, TSLIM axial plane image showing the tissue response surrounding a mid-depth electrode (blue arrow; see online version for color). Contact of the fibrosis has been made with the inner endosteal layer of the RWM (dotted red line). The modiolus, denoted by yellow star, has been contacted between 60 and 90 degrees. Other electrode positions are shown as: B, intrascalar; C, modiolar contact; D, Basilar membrane contact. The rate of contact to each of these structures is shown in table format beneath each corresponding figure. RWM indicates round window membrane; TSLIM, thin-sheet laser imaging microscopy.](image1)

<table>
<thead>
<tr>
<th>0° : RWM Fibrosis</th>
<th>30-60° : Intrascalar</th>
<th>60-90° : OSL/mod contact</th>
<th>90-150° : BM contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow</td>
<td>8% (1 of 13)</td>
<td>85%</td>
<td>31%</td>
</tr>
<tr>
<td>Mid</td>
<td>25% (3 of 12)</td>
<td>83%</td>
<td>67%</td>
</tr>
<tr>
<td>Deep</td>
<td>29% (4 of 14)</td>
<td>86%</td>
<td>79%</td>
</tr>
</tbody>
</table>

![FIG. 5. CAP threshold shift obtained from before surgery and after 28 days for all tested frequencies. Animals are arranged by pretreatment (symbols) and insertion depth (rows). Error bars: SEM. CAP indicates compound action potential.](image2)
human temporal bone studies (31–34) was successfully replicated in the present study.

**Depth, Fibrosis, and Hearing Loss**

In untreated cochleae, the extent of intracochlear fibrosis was found to be directly related to the depth of insertion. The level of fibrosis was found to increase proportionally with the depth of insertion, not only in terms of total volume, but also the extent of scala tympani involved. Previous studies using whole-specimen analysis of guinea pig cochleae found volumetric tissue responses in the range of 0.5 to 1.0 mm$^3$ (26,35), which is comparable to the volume of fibrosis found with the deepest insertion group. An interesting observation among control animals is that while overt trauma to the cochlear walls was almost exclusively limited to the cochleostomy and hook region (irrespective of insertion depth), the total volume of fibrosis increased linearly with insertion depth. This result suggests that the extent of inflammation elicited within the cochlea, which presumably leads to the fibrotic reaction, grows in proportion with the depth of the electrode insertion.

Two regions where the tissue response occurred irrespective of the depth of insertion were retrograde and anterograde (distal) to the location of the electrode array. Retrograde RWM scarring arose despite the introduction of the electrode via a cochleostomy, which has been previously reported in the temporal bone of a hearing preservation implant recipient (36). RWM fibrosis was also associated with low frequency hearing loss of more than 15 dB and this has been previously demonstrated with application of an adhesive to the round window (37) and after implanting CI electrodes through the RWM (22). A possible mechanism of hearing loss is from an increase in cochlear impedance (37), although concurrent hook region fracture that was present in half of all animals with RWM scarring may be a confounding factor. Anterograde (distal) extension of the tissue response beyond the tip of the electrode was found to occur in several animals of deep and mid-depth insertions. Anterograde extension has been previously shown experimentally (10) and in the temporal bone of an implant recipient (36). Since tissue response did not extend beyond the basal turn, it is unlikely to directly account for low frequency hearing loss.

**Steroid Effect**

In steroid-treated animals there was a relatively constant volume of fibrosis across insertion depths, while in control animals the volume of fibrosis increased with insertion depth. Steroids were also found to reduce fibrosis at more apical regions, where contact with the basilar membrane and lateral wall was the commonest type of injury. It is interesting to consider how steroids might influence the volume of fibrosis. Without steroids, the volume of the tissue response does not change significantly between 24 hours and 3 months after surgery (35). This suggests that the clot, which forms in the first minutes after implantation, is a scaffold into which fibroblasts migrate to effect fibrosis (38). A single pre-operative dose of systemic steroid could directly reduce clot formation by potentially stabilizing the blood-labyrinthine barrier or by decreasing the exudation of protein and clotting factors into scala tympani (39). The results of the present study are consistent with this interpretation such that fibrosis was reduced deep in the cochlea, away from regions affected by direct trauma to the scalar walls, and where fibrin would likely have entered scala tympani via an increase in vascular permeability. This explanation might also account for the small or absence effect on fibrosis when a single dose of steroids is administered locally to the perilymph (14,15,18,40).

A steroid effect was only apparent in the hook region and in proximity to the cochleostomy when animals with significant cochlear trauma were excluded. We propose that when there is bleeding into scala tympani, as might be expected with a fracture of the OSL or injury to vessels around the site of the cochleostomy, that preoperative steroids would likely have little effect upon clot formation. In this situation, steroids might reduce the inflammatory response and slow the entry of reparative cells into the cochlea. But it seems unlikely that a single dose of steroids would affect the ingress of fibroblasts into the clot, as this begins towards the end of the first week after surgery (41). These considerations may explain why fibrosis, in the present study, remained exuberant around the OSL and cochleostomy when traumatic insertions had occurred.

The efficacy of steroid-eluting cochlear implant electrodes in reducing fibrosis (13,26) suggests that steroids can act via other mechanisms as well. Under these circumstances, there is no drug present in the cochlea at the time of electrode insertion and so there can be no drug effect upon the immediate inflammatory response and clot formation. We speculate that with steroid elution, there is a slowing of the migration of fibroblasts into the clot, which instead undergoes fibrinolysis.

In conclusion, the amount of fibrosis and, to a lesser extent, postimplantation hearing loss increased proportionally to the depth of insertion. Steroid-mediated effects on the cochlea following implantation were dependent upon the extent and type of cochlear injury. Systemic steroids were most effective with deeper insertions that caused greater cochlear inflammation and in the context of specific pathologies, such as basilar membrane contact. Contrary to our expectations, steroids were less effective in the presence of severe cochlear trauma. The results of this study indicate that a detailed histopathological analysis is required to interpret further experimental findings in this field, particularly when atraumatic techniques are used and there is minimal postimplantation hearing loss.

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Statistical Consulting Centre (University of Melbourne) for guidance on statistical analysis; and, Sjaak Klis (University Hospital Utrecht, Netherlands) for providing the gold ball.

REFERENCES


4.3 Additional Methods

4.3.1 Cochleostomy position

Amira’s spline tool was used to determine the distance of the cochleostomy from the round window. The midpoint of the round window and cochleostomy were both determined by the intersection of two perpendicular views. The Amira spline tool was then used and converted to distance (in mm).

4.3.2 Additional statistical analysis

Spearman-Rho non-parametric correlation tests was performed between cochleostomy position and hearing loss, trauma grade and tissue response.

4.3.3 Glucocorticosteroid dose

Previous work by the Department of Otolaryngology of the University of Melbourne demonstrated efficacy for high- (2 mg/kg) dose dexamethasone over low- (0.2 mg/kg) dose dexamethasone and control groups (Connolly et al., 2011). References to ‘high-dose’ in the above Otology & Neurotology Publication refer to this specific dose of 2 mg/kg.

4.4 Additional Results

4.4.1 Cochleostomy position

The mean linear distance of the cochleostomy from the mid-point of the RWM was 0.90 mm (±0.05 SEM). When considering all cohorts, the cochleostomy position was not correlated with hearing loss, trauma grade nor tissue response (Table 4.1).

<table>
<thead>
<tr>
<th>Hearing Loss</th>
<th>Trauma Grade</th>
<th>Tissue Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (RWM to CO)</td>
<td>-0.156</td>
<td>-0.393</td>
</tr>
</tbody>
</table>

Table 4.1 Non-parametric correlation co-efficients between distance of the round window membrane (RWM) to the cochleostomy (CO) (in mm), 4-week hearing loss,
grade of trauma on the Eshraghi scale (Eshraghi and Van de Water, 2006) and volume of tissue response. No correlations were statistically significant.

Cochleostomy position was also compared between the 8 animals with a RWM tissue response (involving more than 5% of the inner RWM surface) and those without any RWM tissue response. Animals with RWM scarring tended to have a closer positioned cochleostomy (0.81 mm ± 0.07 mm SEM, compared to 0.90 mm ± 0.05 mm SEM), however, this result was not statistically significant (p = 0.287, Student’s t-test).

4.5 Additional Discussion

These results showed cochleostomy position relative to the RWM could be precisely controlled for by the surgeon. For example, the standard error of the mean for cochleostomy distance between animals was 0.05 mm, which was only 5% of the baseline mean (0.90 mm). Furthermore, no relationship between cochleostomy location, hearing loss or surgical trauma was established. This analysis was performed as an accompaniment of the findings from cochlear modelling in Chapter 3, whereby cochleostomies placed closer to the RWM had lower predicted force profiles in radial and axial planes. Studies that wish to specifically explore the location of the cochleostomy would need greater variance in the location to determine if there is any effect on post-implantation hearing loss.
Chapter 5: Intraoperative Monitoring in Cochlear Implantation Surgery
Chapter 5: Intraoperative Monitoring in Cochlear Implantation Surgery

5.1 Introduction

There were several limitations of the graded trauma model developed in Chapter 4. Altering the depth of insertion of a highly flexible electrode did not correlate with an increase in trauma nor post-implantation hearing loss. For example, intracochlear trauma was mostly confined to the hook region and was found to occur irrespective of the depth of insertion. Additionally, lower frequency hearing losses tended to occur with deeper insertions than the shallowest insertion, but this trend was neither significant nor clinically meaningful (with less than 5 dB of hearing loss for most groups and frequencies). Collectively, these results show that a different model of surgical trauma is required to investigate its role with post-implantation hearing loss.

A further finding from Chapter 4 requiring additional exploration was the absence of a glucocorticosteroid (‘steroid’) effect on CAP threshold shifts. A threshold shift reflects a loss of sensitivity to weak sounds and thresholds derived from the audiogram have been increasingly shown to be poor predictors of some types of auditory deficits, such as noise exposure and aging (Plack et al., 2014). Thresholds are largely influenced by the function of the outer hair cells; for example, almost 80% of inner hair cell loss may occur before measurable changes in threshold are affected (Lobarinas et al., 2013).

The magnitude (also termed amplitude) of the CAP and CM responses have shown promise as useful recording paradigms for patients undergoing hearing preservation CI surgery (Campbell et al., 2016). The CAP magnitude directly reflects the number of auditory nerve fibres firing (Ferraro and Durrant, 2006) but also inner hair cell output, since afferent fibres of the auditory nerve primarily innervate inner hair cells (Spoendlin, 1966). Recently, Reiss et al. (2015) showed a deterioration in response magnitudes occurred with little to no change in threshold after CI in guinea pigs. Additionally, declines in the response magnitude were correlated with losses in pre- and post-synaptic terminals of hair and spiral ganglion cells, termed synaptopathy (Reiss et al., 2015). Limited hair cell loss was also observed, even in cochlear regions with considerable threshold shifts. Additionally, Ye et al. (2007) showed that topical steroid administration improved short-term hearing amplitudes but not threshold
in guinea pigs following implantation. Hence, the aim of this study was to determine if the efficacy of systemic steroid administration on post-implantation hearing loss, measured by CAP amplitude, is altered by surgical trauma. Recording configurations in Chapter 4 were modified for this study to include peak-to-peak amplitude calibrations, equal to the dB HL scale, to derive amplitude shifts (along with thresholds).

Since altering the depth of insertion with a flexible electrode that incorporated contemporary human electrode designs (in Chapter 4) failed to produce a reliable model of graded trauma (or hearing loss), electrode stiffness was trialled as a trauma model for this Chapter. Ex vivo implantation studies have shown stiffer electrodes to have higher frictional forces (Nguyen et al., 2012) and to be more traumatic than flexible electrodes (Briggs et al., 2001, Adunka et al., 2004). However, potential relationships between electrode stiffness, surgical trauma and long-term post-implantation hearing loss have only recently been explored in animal studies. Giordano et al. (2014) showed a stiffer electrode caused greater threshold shifts than a softer electrode. Similarly, Drouillard et al. (2017) found a stiffer electrode (by a factor of 7) caused significantly poorer hearing. However, these studies have lacked a detailed analysis of the electrode path and intracochlear trauma. For this study, the guinea pig electrode from Chapter 4 was modified to reduce the risk of design variability by reducing the number of rings (from 3 to 1) and using a single straight electrode wire (compared to helical wires in Chapter 4). The stiffness of the electrode was altered through embedding in one of two medical grade silicones (hard or soft). Next, these electrodes were validated in an epoxy cochlear model for their insertion force and depth. By controlling for cochleostomy position (from Chapter 3) and depth of insertion (from Chapter 4), electrodes were then implanted into the cochleae of guinea pigs. Electrode flexibility was compared with surgical trauma and hearing loss.

A secondary aim of this study was to determine if intraoperative monitoring could predict surgical trauma and post-implantation hearing loss. The overriding goal of intraoperative recordings is to have reliable method of monitoring the electrode’s intracochlear behaviour so that hearing preservation manoeuvres can be performed. Two promising intraoperative monitoring techniques are cochlear implant-based ECochG and the force of the electrode insertion. For implant-based ECochG, measuring ECochG amplitude is the most practical method since thresholds lack the instantaneous, real-time feedback. Preserving the amplitude of the CAP (Mandala et al., 2012) and the CM (Acharya et al., 2016, Campbell et al., 2016)

*Chapter 5: Intraoperative monitoring*
have shown promise in predicting the extent of hearing preservation. However, ECochG recordings have mostly arisen from observational studies that are yet to provide meaningful interpretations for surgeons performing cochlear implantation.

The magnitude of electrode insertion force as a feedback mechanism has been shown in an animal model to correlate with gross structural damage (Wade et al., 2014). However, Drouillard et al. (2017) recently found maximal peak insertion force did not correlate with hearing loss after implantation. This result may be explained by a lack of sensitivity or accuracy arising from difficulties measuring the relatively small force of electrode insertion from a sensor placed beneath the head or from variabilities of surgery. To address this secondary aim of the study, a hand-held insertion tool was designed and implemented for performing force measurements. Furthermore, a new multi-tone stimulus was developed for intraoperative ECochG so that CAP and CM amplitudes could be simultaneously performed. Intracochlear ECochG was recorded from the electrode ring at the tip of the electrode array.

The hypotheses tested in this study were that:

i) Flexible electrodes cause less trauma and post-implantation hearing loss

ii) Systemic steroids reduce post-implantation hearing loss, as measured by an amplitude shift, in the presence of minimal trauma only

iii) An irreversible decline in the intraoperative ECochG (either CAP or CM) amplitude is correlated with post-implantation hearing loss and grade of surgical trauma

iv) A rise in the intraoperative force recording is correlated with post-implantation hearing loss and grade of surgical trauma

5.2 Hearing Research Publication
Intraoperative force and electrocochleography measurements in an animal model of cochlear implantation


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ABSTRACT
To preserve residual hearing, techniques for monitoring and reducing the effects of trauma during cochlear implant surgery are being developed. This study examines the relationships between intraoperative recordings (electrode insertion force and electrocochleography), trauma, and hearing loss after cochlear implantation. The study also evaluated the efficacy of intravenous steroids for reducing hearing loss after implantation. Thirty-two normal-hearing guinea pigs were randomly implanted with electrode arrays of differing stiffness ('hard' or 'soft'). These arrays used an intracochlear electrode to record electrode insertion force and electrocochleography responses to a multi-frequency acoustic stimulus during implantation. Additionally, sub-cohorts of animals were administered intravenous saline ('control') or dexamethasone ('steroid') prior to surgery. Subsequent hearing loss was assessed using electrocochleography recordings from the round window membrane prior to surgery and 4 weeks after implantation. After 4 weeks, cochleae were harvested and imaged with thin sheet laser imaging microscopy. After 4 weeks, compound action potential (CAP) thresholds did not differ between steroid and control groups. The CAP amplitude at low-mid frequencies decreased after implantation with a hard electrode, an effect which was partly negated by administering steroids. A decrease in the 'intraoperative' CAP amplitude preceded the reporting of insertion resistance by the surgeon by 5.94 s (±4.03 s SEM). Intraoperative CAP declines were also correlated with higher grades of trauma (r = 0.56, p < 0.01) and greater hearing loss (r = 0.56, p < 0.01). This relationship was not repeated with intraoperative cochlear microphonics. A rise in intraoperative force, which preceded the reporting of resistance by 0.71 s (±0.15 s SEM), was correlated with trauma (r = 0.400, p = 0.04) but not hearing loss (r = 0.297, p = 0.27). Preserving intraoperative CAP amplitudes during implantation was predictive of an atraumatic insertion and reduced post-implantation hearing loss. A rise in force usually preceded the reporting of resistance, although by less than 1 s. These results suggest that intraoperative CAPs may offer a more robust feedback mechanism for improving hearing preservation rates than cochlear microphonic and electrode insertion force recordings, especially considering the rapid changes in insertion force and relatively slow human reaction times. Pre-operative steroids were effective in reversing loss of CAP amplitude with hard electrodes and evoked by lower frequency tones, which suggests a possible role in reducing synaptopathy.

1. Introduction
Preserving residual hearing to facilitate ‘electroacoustic stimulation’ (EAS) is now a widely recognised goal of cochlear implantation surgery. EAS combines electric and acoustic hearing in the same ear and is particularly beneficial for perceptual tasks relying on pitch resolution, such as music appreciation and speech perception in background noise (Gantz et al., 2005; Santa Maria et al., 2013). Complete or partial preservation of residual hearing may be achieved by minimizing electrode insertion trauma.
(Eshrarghi and Van de Water, 2006; O'Leary et al., 2013), and this has led to the exploration of intraoperative techniques that monitor the behaviour of the electrode.

Two potential intraoperative monitoring techniques are electrococleography or ECochG (the electrical response of the cochlea to acoustic stimulation) and the force of the electrode insertion. Preserving the amplitude of electrococleography responses like the compound action potential (CAP) (Mandala et al., 2012) and the cochlear microphonic (CM) (Acharya et al., 2016; Campbell et al., 2016) have shown promise in predicting the extent of hearing preservation. However, ECochG recordings have mostly arisen from observational studies that are yet to provide meaningful interpretations for surgeons performing cochlear implantation. Although recent findings for intracochlear measurements made both in animals (Choudhry et al., 2014; DeMason et al., 2012) and in humans with scala translocation have shown ECochG to be predictive of some types of surgical trauma (O'Connell et al., 2017).

The magnitude of electrode insertion force as a feedback mechanism is widely believed to correlate with structural damage (Wade et al., 2014) and by extension, the level of post-implantation hearing loss (Kobler et al., 2015). However, these assumptions are yet to be explored experimentally because of difficulties measuring the relative relation of electrode insertion force to sensor placed beneath the head (Miroir et al., 2012). Recent, insertional forces measured along the axis of the electrode have been shown to be similar to a conventional 6-axis sensor placed beneath a cochlear model (Miroir et al., 2012) and this may provide a more practical option for intraoperative recordings.

The purpose of this animal-based study was to determine correlations between intraoperative cochlear implant-based ECochG, electrode insertion force recordings and post-implantation hearing loss. Two electrode arrays of differing stiffness were validated in an epoxy cochlear model for their insertion force and depth, and then implanted into the cochleae of guinea pigs. A hand-held insertion tool was designed and implemented for performing uni-axis force measurements. Systemic steroids were administered pre-operatively in a placebo controlled manner to assess their relative efficacy (Connolly et al., 2011; Lee et al., 2013; Rah et al., 2016) in reducing the effects of specific types of electrode insertion trauma (Lo et al., 2017b). Correlations between steroid pre-treatment, the level of surgical trauma and post-implantation hearing loss were examined.

2. Materials and methods

2.1. Cochlear implant array

The cochlear implant was a single channel electrode array, 15 mm in length and with a single platinum ring at its tip. This ring was welded to a straight internal platinum wire 25 μm in diameter. The ring and internal wire were embedded in a silastic carrier comprised of one of two types of silicone: hard (MDX4-4880 Dow Corning Products, USA) or soft (MDX4-4210 Dow Corning Products USA). Both electrodes used undyed materials and did not differ in their appearance. The maximum diameter of the implant was 0.45 mm, narrowing to 0.41 mm towards the tip, which models the tapering and tip diameter of contemporary human electrode designs (Mukherjee et al., 2012). At the basal end of the electrode array, the internal platinum wire was welded to a platinum contact that ran perpendicular to the array (Fig. 1). This enabled intracochlear ECochG recordings from the ring electrode at the tip of the array to be conducted to the platinum contact that remained outside of the cochlea.

2.2. Replica model force measurements

Force measurements on the two electrode designs were performed by advancing electrodes attached to a load cell in to a guinea pig scala tympani model. Firstly, a 3-D epoxy model of the basal turn was created by molding a silicone cast in crystal epoxy resin (Fig. 2). The silicone cast of scala tympani was produced by dislocating the stapes to open the oval window, followed by the injection of silicone through an opening in the round window (RW). After hardening, the silicone was removed to produce a caste of a full basal turn of scala tympani, commencing at the RW. This segment was molded in crystal epoxy resin and the silicone was then withdrawn. A cochleostomy was drilled into the epoxy model by an experienced surgeon using a 0.8 mm diamond bur and after anatomical orientation.

Insertion forces were recorded with an Instron 5543 Force Measurement System (Instron; Deutschland GmbH, Pfungstadt, Germany). The scala tympani (ST) model was attached to the lower gripping jaw and oriented with the cochleostomy and insertion axis directed vertically. The upper gripping jaw, connected to the load cell, was used to fix the test electrode and direct it downwards into the model. Four electrodes made of hard or soft silicone (2 each) were inserted twice in to the scala tympani model. The model was filled with normal saline and electrodes were pre-inserted into the opening of the cochleostomy. Electrodes were inserted at a constant speed of 15 mm/min (Rajan et al., 2013) until buckling of the basal portion of the electrode was observed. Force measurements were captured every 4 ms and all insertions were video recorded (see Appendix 1). The angular depth of insertion was determined as degrees of rotation around the modiolus, commencing at the midpoint of the RW and finishing at the electrode tip (Lo et al., 2017a). These depths were analysed from still photographs (Fig. 2) in the axial orientation (Lo et al., 2017a) using the 2D angle tool in Amira 5.4 (Visualization Sciences Group, France).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.heares.2017.11.001.

2.3. Hand-held insertion tool

A hand-held insertion tool (Fig. 1) was developed that was based upon a previous instrument capable of sensitive and accurate force recordings (Miroir et al., 2012). During insertions, the electrode array pushed a blunt pin into a dextran-filled tube comprised of carbon fibre (Engineering Supplies, QLD, Australia). Dextran fluid and carbon fibre tubing were chosen to lower frictional forces (Kontorinis et al., 2011; Miroir et al., 2012). Abutting the other end of the blunt pin to the electrode was an uni-axis force sensor (milli-Newton force sensor, EPFL, Lausanne, Switzerland). The force sensor has a range of 0–0.4 N, with 4 mN resolution, and has been shown to record similar measurements to a conventional 6-axis sensor placed beneath a cochlear model (Miroir et al., 2012). Sensor data was recorded at a sampling rate of 1000 Hz and acquired with a DT9847 (Data Translation GmbH, Germany) USB data acquisition. Before every fifth implantation, the force sensor was re-calibrated using 1.5-, 5- and a 10-g weights.

2.4. Experimental procedure

All procedures in this study were approved by and performed in accordance with the Animal Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (project 191335AU). Thirty-two adult Dunkin-Hartley tricolour guinea pigs, weighing >400 g, were used in this study. Animals underwent left sided cochlear implantation with one of two electrode types (hard or soft) after receiving either intravenous dexamethasone (‘steroid’) at 2 mg/kg
or saline (‘control’) 60 min prior. One surgeon performed all surgeries and was blinded to the intravenous treatment and electrode type. All researchers were blinded to the treatment groups for hearing and TSLIM analysis. All animals received a standardised drug administration and implantation procedure (Lo et al., 2017b). In brief, saline or high-dose systemic dexamethasone sodium phosphate (2 mg/kg; Sigma-Aldrich, USA) was administered into the right jugular vein prior to the commencement of the implant surgery (Connolly et al., 2011; Lee et al., 2013). Anaesthesia for surgical procedures and ECoChG recordings was inducted and maintained with intramuscular ketamine (60 mg/kg, Troy Laboratories Pty, Ltd, Sydney, Australia) and xylazine (4 mg/kg, Troy Laboratories Pty Ltd). The local anaesthetic lignocaine (1 mg/mL, Troy Laboratories Pty Ltd) was injected prior to a post-auricular incision. A left sided bullotomy was performed with a scalpel, followed by creation of a tr using a 0.8 mm diamond bur. The cochlear implant was inserted at a constant slow speed (approximately 15 mm/min) to the depth of the 4 mm plug or to the point of first resistance, whichever came first. The hand-held tool was used for the insertion while simultaneously recording force and ECoChG. The duration of the insertion was recorded.

2.5. Acoustic stimuli and recording configurations

Two distinct ECoChG configurations were used for long-term hearing results (‘extracochlear’) and during implantation surgery (‘intraoperative’). A multi-tone stimulus was used to determine CAP and CM amplitudes from the implant during intraoperative ECoChG, while pure tones were used to determine extracochlear CAP thresholds performed prior to cochleostomy and at the end of the survival period (at 4-weeks).

Extracochlear RW recordings have been previously described (Lo et al., 2017b). In summary, acoustic stimuli were delivered via a loudspeaker (Richard Allen DT-20) placed 0.1 m from the test ear. The stimuli for RW ECoChG recordings were computer generated tone pips of 20 ms duration with 1 ms linear onset/offset ramps, presented at 2, 4, 8, 16, 24 and 32 kHz. These were presented every 50 ms and averaged over 25 repetitions. Responses were pre-amplified by a factor of 1000 (DAM-5A, W-P Instruments) and then band-pass filtered (Krohn-Hite 3750) at 0.1 Hz–100,000 kHz. The responses were digitised by a 24-bit analogue-to-digital converter (DT9847S) that sampled at 216 kHz. Alternating stimuli with condensation and rarefaction phases were saved separately, allowing them to be added to derive a CAP. CAP traces were then digitally filtered between 10 and 1500 Hz. Hearing thresholds were visually inspected by a single blinded investigator in 5 dB decrements and determined as corresponding to the minimal intensity for which a CAP was clearly recognised (Lo et al., 2017b). The maximum output plus 5 dB was recorded if no responses were present. The recording noise floor was determined by measuring waveforms for the average of 240 recordings to the lowest acoustic input and without ECoChG traces. RW ECoChG recordings were performed with a gold ball 0.5 mm in diameter placed on the RW and a subcutaneous needle electrode placed on the nape of the neck. At the end of the survival period, the wound was reopened and the gold ball electrode was again placed on the RW for final recordings. An illustrative dataset of CAP responses to an 8 kHz tone is shown in Fig. 3.
Fig. 3. Compound action potential (CAP) traces from the round window in response to an 8 kHz tone recorded before (A) and 4 weeks after (B) cochlear implantation. Threshold corresponded to 25 dB (A) and 40 dB SPL (B). T = 0 ms corresponds with the first change in sound pressure at the external ear canal. Scale bar = 100 mV.
During implantation, intracochlear ECochG was recorded from the electrode ring at the tip of the array. Intracochlear ECochG was presented as a multi-tone stimulus of alternating polarity consisting of 2.2, 3.8 and 8.0 kHz tone pips of 12 ms duration, presented simultaneously at a rate of 12 per second and with rise/fall times of 1 ms. These stimuli were chosen to avoid the interference of secondary harmonics and to maintain an equal power distribution across the frequencies. 8 kHz has a frequency place mapping originating from the upper basal turn of the guinea pig, while 2 and 4 kHz are located towards completion of the first turn and in the second turn, respectively (Greenwood, 1990). The stimuli were delivered continuously (with a 50 ms interstimulus interval) at 85 dB HL and via EARTONE-3A insert tube phones (Aearo Technologies, USA). The stimulus intensities were calibrated with peak-to-peak amplitudes equal to the dB HL scale. The electrode insertion commenced after a minimum of 10 s of stable ECochG and baseline electrode insertion force recordings. The amplitude of the CAP was measured from the first negative peak (N1) to the subsequent positive peak (P1) of the waveform between 1 and 3 ms on the CAP trace.

### 2.6. Thin sheet laser imaging microscopy

Four weeks after implantation surgery, and after follow up ECochG recordings, guinea pigs were transcardially perfused with 0.9% saline followed by 10% neutral buffered formalin. Cochleae were then removed and prepared for thin sheet laser imaging microscopy (TSLIM) as previously reported (Lo et al., 2017a). In brief, cochleae underwent decalcification in 10% ethylenediaminetetra-acetic acid for 3 weeks, dehydration by immersion in a graded ethanol series (50%, 70%, 90%, 100% and 100% each for 24 h) and clearing with Spalteholz fluid (5:3 of methyl salicylate: benzyl benzoate; Sigma-Aldrich, USA). TSLIM generated high-resolution images (Lo et al., 2017a) that were reconstructed in 3D and replica ST model are shown in (Lo et al., 2017b). The level of trauma was graded per the scale reported by Eshraghi et al. (Eshraghi and Van De Water, 2006): 1 – no observable macroscopic trauma; 2 – elevation of the basilar membrane; 3 – translocation of electrode to scala vestibuli; 4 – fracture of the osseous spiral lamina (OSL) or modiolus). Traumatic insertions were deemed to have scored >1 on the Eshraghi scale in any cochlear region. A cochleotopic map was determined by passing a spline along the tunnel of Corti and then mapping length along this line to Wada’s equation (Wada et al., 1998), adapted from Greenwood’s equation (Greenwood, 1990).

### 2.7. Statistical analysis

Statistical analyses were performed with SPSS version 23.0 (IBM). Spearman-Rho non-parametric correlation tests and Student t tests were performed where appropriate. For parametric data, analysis of variance (ANOVA) was used. When repeated-measures were analysed, ANOVA was applied with post-hoc testing using a Bonferroni correction for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

### 3. Results

#### 3.1. Force measurements from a replica model

Force and insertional depth measurements made with the Instron and replica ST model are shown in Table 1. A lower peak force was seen with the soft electrode compared to the hard electrode ($p = 0.01$, Student’s t-test). However, a shallower insertion depth was achieved with the soft electrode compared to the hard electrode (Table 1). Consequently, the electrode was modified for the cochlear implantation experimental procedure with the inclusion of a plug at 4 mm to control for the depth of insertion (Fig. 1).

#### 3.2. Cochlear implantation surgery

The characteristics of the electrode insertion performed in vivo using the hand-held insertion tool are shown in Table 2. Significantly lower peak forces were recorded with the soft electrode than the hard electrode ($p = 0.01$). Overall, rates of macroscopic trauma were similar between the electrode types (Table 2), although hard electrodes caused more OSL fracture, while soft electrodes were more likely to elevate the basilar membrane. The electrode tip for all insertions resided against the lateral wall, excluding 4 insertions that had translocated to scala vestibuli. The insertion depths did not differ between electrode types ($p = 0.68$, Student’s t-test) following the inclusion of the 4 mm plug (Table 2). Insertion depths had a cochleotopic place corresponding to approximately 7–11 kHz.

### Table 1

<table>
<thead>
<tr>
<th>Electrode type</th>
<th>Mean peak load, mN (±SEM)</th>
<th>Insertional depth, μm (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard</td>
<td>102.3 (±13.9)</td>
<td>155.9 (±27.7)</td>
</tr>
<tr>
<td>Soft</td>
<td>31.8 (±4.1)</td>
<td>138.6 (±4.4)</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Electrode type</th>
<th>Mean peak force, mN (±SEM)</th>
<th>Insertional depth, μm (±SEM)</th>
<th>BM elevation</th>
<th>OSL fracture</th>
<th>SV translocation</th>
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</thead>
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<tr>
<td>Hard</td>
<td>42.0 (±4.6)</td>
<td>171.6 (±6.7)</td>
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<td>5</td>
<td>2</td>
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<tr>
<td>Soft</td>
<td>27.6 (±2.8)</td>
<td>169.6 (±10.8)</td>
<td>4 (of 13)</td>
<td>2</td>
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</table>

### Table 3

<table>
<thead>
<tr>
<th></th>
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<tr>
<td></td>
<td>Thr</td>
<td>SEM</td>
<td>Thr</td>
<td>SEM</td>
<td>Thr</td>
<td>SEM</td>
</tr>
<tr>
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<td>38.8</td>
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<td>20.6</td>
<td>1.8</td>
<td>6.3</td>
<td>0.8</td>
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<td>2.0</td>
<td>13.8</td>
<td>1.6</td>
<td>4.4</td>
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<tr>
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<td>3.3</td>
<td>17.5</td>
<td>2.8</td>
<td>6.3</td>
<td>2.3</td>
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</tbody>
</table>
3.3. Post-implantation hearing loss and tissue response

The mean (and standard deviation) of the noise floor of the recordings was 0.06 mV (±0.026 mV). Pre-implantation hearing did not differ between cohorts (Table 3). Post-implantation hearing loss, estimated from the RW CAP recordings at 4 weeks, was most apparent in the mid-high frequencies of 8–32 kHz (Fig. 4). For saline-treated animals, hard electrodes had greater mean hearing loss at 8 kHz compared to soft electrodes, although this difference was statistically insignificant ($p = 0.14$, Student’s $t$-test). No difference in CAP threshold was identified between intravenous pre-treatments for either electrode type.

CAP amplitude shifts, defined as the pre-operative minus the post-operative maximum amplitude, are shown in Fig. 5. The average magnitude of the shift was less than 0.25 mV for all treatment groups except saline-treated animals implanted with a hard electrode, where values were higher across all frequencies $p$. On repeated measure ANOVA (with frequency as a within subject variable), a significant effect of steroid treatment on tissue response was identified for hard electrodes ($p = 0.03$). No significant interaction was found between these factors or with the use of soft electrodes. The total volume of tissue response was similar between electrode types and intravenous pre-treatments (Fig. 6).

3.4. Intraoperative recordings

Intraoperative ECochG or electrode insertion force recordings were successfully performed in 30 animals. ECochG recordings were unable to be elicited in six animals even though the stimulus intensity was at least 15 dB above preoperative thresholds. In these instances, hearing loss may have arisen from surgical interventions prior to implantation, such as from the creation of the cochleostomy or the inadvertent entry of blood into the middle ear (Mandala et al., 2012; Radeloff et al., 2007). One animal died during the anaesthesia prior to intraoperative recordings, while a further 4 animals died in the immediate post-operative period. Two examples of intraoperative recordings are shown in Fig. 7 and Fig. 8.

3.5. Correlations for intraoperative recordings, histopathology and hearing

The amplitude of CAP recordings tended to increase after the commencement of the insertion. In some recordings (see Fig. 7), the CAP response was within 10% of its maximum amplitude (6 of 25 animals) by completion of the recording. However, most recordings (19 of 25) had a greater than 10% loss in the CAP amplitude (see Fig. 8). Losses in the intraoperative CAP amplitude preceded the reporting of resistance by the surgeon in 74% of insertions (14 of 19) and by a mean of 5.94 s ($±$ 4.03 SEM). When considering all cohorts, an intraoperative loss of CAP amplitude was associated with higher grades of trauma and greater hearing loss (Table 4). In this series, intraoperative CAP reduction was not correlated with the volume of tissue response.
corresponding to the initial peak in force, along with contact to the lateral wall from when present) preceded the reporting of resistance by a mean of 0.71 s (±0.15 SEM). Since some traces showed multiple peaks during the insertion (Fig. 8), cumulative electrode insertion force was used for correlation analysis. When considering all cohorts, the cumulative force during the insertion was positively correlated with grade of trauma (Table 4). In this series, intraoperative electrode insertion force was not associated with greater tissue response, increased hearing loss, or losses in any of the intraoperative ECochG.

4. Discussion

4.1. Overview of findings

Losses in the intraoperative CAP response correlated with higher grades of trauma and greater post-implantation hearing loss. No such correlation was found for any of the intraoperative CM response. In vivo electrode insertion force recordings closely preceded the reporting of resistance and were often complex, multi-peak traces. The cumulative force of insertion was associated with higher grades of trauma, but not with hearing loss. Steroids were effective in reversing CAP amplitude losses, which was most pronounced in low-mid frequencies.

4.2. Intraoperative ECochG recorded from the cochlear implant

Intraoperative CAP loss was moderately correlated with post-implantation hearing loss at 4 weeks. Interestingly, post-implantation hearing loss did not correlate with intraoperative recordings of electrode insertion force or CM responses. Similarly, clinical studies have shown that intraoperative ECochG losses may be associated with unsuccessful or incomplete hearing preservation (Campbell et al., 2016; Dalbert et al., 2016; Mandala et al., 2012; Radeloff et al., 2012). However, these studies have been largely in the presence of traumatic insertions, including scalar dislocation (Dalbert et al., 2016), kinking of the electrode (Radeloff et al., 2012) and from a fast insertion speed (Mandala et al., 2012).

In the present study, post-implantation hearing loss was not correlated with a higher grade of surgical trauma. A likely explanation is that insertions that have traditionally been considered ‘atraumatic’, and in the absence of gross trauma, can still result in significant hearing loss (Eshraghi et al., 2005; Lo et al., 2017b) from damage occurring at the molecular level. This includes molecular injury to the organ of Corti arising from inflammation (Eshraghi and Van de Water, 2006; Eshraghi et al., 2005), micromechanical changes from contact between the electrode and basilar membrane (Campbell et al., 2016) or from fluid displacement within scala tympani (Harris et al., 2017). It is highly plausible that intraoperative CAP recordings could detect some of these molecular or micromechanical causes of hearing loss, in addition to more traumatic causes, and this would explain the strong correlation we
found with post-implantation loss.

In patients with residual hearing, the CM recorded from an intracochlear electrode has been found to be reliable (Campbell et al., 2015) and more useful predictor of post-implantation hearing loss than the CAP (Campbell et al., 2016). This contrasts with our findings that the CAP was the better predictor of post-operative hearing and we suspect this difference arises from our use of a novel multi-tone acoustic stimulus. Previous studies have used a pure-tone as the acoustic stimulus, whereas the multi-tone stimulus we used originated from basal, first and second turns of the guinea pig cochlea. In the present context, a reduction in CAP amplitude (measured from the intracochlear electrode) likely represented a decline in cochlear function from any of the three sites generating the summed acoustic response. As such, intraoperative CAP responses were found to be more sensitive to cochlear trauma than localised CM responses that reflect hair cell activity in the vicinity of the electrode only (Campbell et al., 2017). By way of contrast, when a low frequency pure acoustic tone is used as the stimulus (as in previous studies) the CM in most patients grows as the electrode is advanced into the cochlea and approaches the cochlear site generating the response (Bester et al., 2017). The observations in this study raise the intriguing possibility that the CM may not be the ideal cochlear potential to record when both are accessible. In practice however, monitoring of the CAP is not reliable in humans because the CM is more robust than the CAP, and in many patients the latter cannot be identified at all (Campbell et al., 2015). Whether this reflects neural dysfunction in humans with hearing loss or differences in the anatomy between species is not yet understood.

4.3. Intraoperative force

A sudden rise in electrode insertion force measured from the uni-axis sensor nearly always preceded the reporting of resistance by the surgeon. This means that monitoring for a rising intracochlear force can detect surgical resistance earlier than by touch alone. However, force was not found to be as sensitive as intracochlear ECoG in detecting a degradation in cochlear function, which is supported by a recent animal study (Drouillard et al., 2017). In this study, where the insertion speed was 15 mm/min, a 1 s latency in detecting resistance would mean the electrode travelled approximately 0.25 mm further from the force change than by the time the surgeon stopped the insertion. The electrode would have advanced less, and likely caused less trauma, if the speed of insertion was slowed, and vice versa if increased.

Ideally, these findings should be confirmed by conducting a trial where the surgeon is given an opportunity to alter their operative technique in response to real-time feedback on the force or CAP recordings. Intraoperative electrode insertion force recordings showed considerable variance within electrode types, despite best efforts to control for the surgical technique. Subtle differences in the placement of the cochleostomy have been shown to greatly alter the angle of insertion and point of first contact in the tightly coiled guinea pig cochlea (Lo et al., 2017a), which may have contributed to the results observed. Lower forces were found with the experimental procedure compared to the scala tympani model, especially for hard electrodes. This likely arises from differences in the friction forces with live tissue compared to the epoxy model. From reviewing video footage, we suspect that hard electrodes may not use insertion forces to overcome the point of impingement from first contact with the wall of the scala tympani model (Zhang et al., 2010). The soft silicone electrodes appeared to have less impingement in the scala tympani model than hard electrodes.

4.4. Steroid effect

Systemic steroids did not affect the total volume of fibrosis. A previous study using the same surgical technique, including cochleostomy position and speed of insertion, did identify a steroid-mediated effect on tissue response (Lo et al., 2017b). However, this effect was greatly diminished for traumatic insertions (Lo et al., 2017b), which may explain the absence of a steroid effect in the present study, considering that a much higher rate of trauma was encountered (28% for insertions with an Eshraghi score >1, compared to our previous study of 10%). Highly traumatic insertions, such as with a fracture of the OSL, result in the entry of blood (and subsequent clot formation) in scala tympani. This is accompanied by the ingress of fibroblasts into the clot towards the end of the first week after surgery (Kel et al., 2013), and the results of the current study and Lo et al. (2017b), suggest that a single dose of steroids is unlikely to affect this process. The increased rate of trauma in the present study likely arose from the use of a straight platinum wire, when a helical designed wire was used in the previous study to model contemporary electrodes (Lo et al., 2017b).

For high frequency hearing, no steroid effect was found on CAP amplitude or threshold. For low-mid frequency hearing, steroid administration helped to maintain CAP amplitudes (that otherwise fell in response to suprathreshold acoustic stimulation), but had no effect on threshold. This has been previously demonstrated by Ye et al. (2007), and has recently been attributed to synaptopathy. Reiss and colleagues found that a reduction in CAP amplitude growth in response to low frequency sounds correlated with losses in the pre- and post-synaptic terminals of hair and spiral ganglion cells (Reiss et al., 2015). Although TSLIM did not allow us to count synaptic ribbons, our results are consistent with this interpretation, which leads to the tantalizing possibility that synaptopathy, if present, may be amenable to steroid treatment. Previous studies have shown that in experimental cochlear implantation, steroids act directly on inner hair cells through anti-inflammatory, anti-apoptotic and antioxidant pathways (Dinh et al., 2008; Haake et al., 2009; van de Water et al., 2010).

4.5. Conclusion

Preserving the intraoperative CAP in response to a simple multi-tone stimulus was predictive of an atraumatic insertion and reduced post-implantation hearing loss. No relationship was found for intraoperative CM responses. Intraoperative electrode insertion force recordings were correlated with the grade of trauma, but not with hearing loss. Losses in the intraoperative CAP and force spikes usually preceded the reporting of resistance by the surgeon, although by less than 1 s for electrode insertion force measurements. The latency of the human response to an increase in force is one factor that should be considered in determining the speed of electrode insertion. These results suggest that an intraoperative, intracochlear real-time recording of a global ECoG response, such as a multi-tone CAP, may offer a more robust feedback mechanism for improving hearing preservation rates. It was also demonstrated that steroids were effective in preserving low frequency CAP amplitude losses, which may have important implications for EAS.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heares.2017.11.001.

References


5.3 Additional Methods

5.3.1 Cochlear implant array – addendum

For this study, the guinea pig electrode from Chapter 4 was modified to reduce the risk of handling and design variability. Since flexibility was the controlled variable of interest, all obsolete components of the electrode were therefore removed. Only one electrode ring at the tip was required for performing intraoperative ECochG. Therefore, only a single channel electrode array was used, instead of the three-channel electrode array in Chapter 4. Changes in stiffness along any segment of the array can alter the handling and tactile feedback of the implant (Jolly et al., 2010). As such, it was important that electrodes in this study had consistent flexibility along their entire length. For this reason, a straight platinum wire was used instead of the helical design in Chapter 4.

5.3.2 Replica model force measurements - addendum

Force measurements on the two electrode designs (hard and soft), along with the ‘atraumatic’ electrode developed in Chapter 4, were performed on a 3-D epoxy model of the basal turn of the guinea pig. Silicone was injected through the RWM of a freshly harvested guinea pig cochlea to create a caste of scala tympani (Figure 5.1). After hardening, the silicone was removed to produce a caste of scala. This was trimmed to include just the basal turn and covered in a water-based lubricant before moulding in crystal epoxy resin. The silicone was subsequently withdrawn.

Figure 5.1  Left, guinea pig cochlea with dislocated stapes. Right, silicone cast of scala tympani of the same cochlea that was later moulded in crystal epoxy resin.

Chapter 5: Intraoperative monitoring
Insertion forces were recorded with an Instron 5543 Force Measurement System (Instron; Deutschland GmbH, Pfungstadt, Germany). The scala tympani (ST) model was attached to the lower gripping jaw and oriented with the cochleostomy and insertion axis directed vertically (Figure 5.2). The upper gripping jaw (red star in Figure 5.2), connected to the load cell, was used to fix the test electrode and direct it downwards into the model. All insertions were video recorded (blue star).

![Intron 5543 Force Measurement System displaying the upper jaw with load cell (red star). The scala tympani model was placed on the lower jaw, directly below. All insertions were video recorded (blue star).](image)

**Figure 5.2** Intron 5543 Force Measurement System displaying the upper jaw with load cell (red star). The scala tympani model was placed on the lower jaw, directly below. All insertions were video recorded (blue star).

### 5.3.3 Cochleostomy

As with 4.3 Additional Methods, Amira’s spline tool was used to determine the distance of the cochleostomy from the round window. The midpoint of the round window and cochleostomy were both determined by the intersection of two perpendicular views. The Amira spline tool was then used and converted to distance (in mm).

### 5.3.4 Statistical analyses

For additional results of correlation co-efficients pertaining to the cochleostomy position, Spearman-Rho non-parametric correlation tests and Student t tests were performed where appropriate. Force measurements of the three electrode designs using the replica model were
compared using analysis of variance (ANOVA) implemented under the Univariate General Linear Model with post-hoc testing using Student t test.

5.4 Additional Results

5.4.1 Force measurements from a replica model – addendum

Mean peak forces of the 3 electrode types from Chapter’s 4 and 5, using the Instron Measurement system, are shown in Figure 5.3. On ANOVA, electrode type was found to affect the peak force. Post-hoc testing revealed that the atraumatic electrode differed significantly from both soft and hard electrodes (p=0.01, Student’s t-test), and with significance difference between soft and hard electrodes (p = 0.01, Student’s t-test).

![Figure 5.3](image-url) Maximal peak force measured during insertion of the three electrode array types. Hard and soft electrodes were used in the present study, while the atraumatic electrode was used in Chapter 4. Values are means, bars are SEM.

5.4.2 Cochleostomy position

The mean linear distance of the cochleostomy from the mid-point of the RWM was 0.86 mm (±0.02mm SEM). When considering all cohorts, the cochleostomy position was not correlated with hearing loss, trauma grade nor tissue response Table 5.1Table 5.1.  

Chapter 5: Intraoperative monitoring
Table 5.1  Correlation co-efficients between distance of RWM to CO, 4-week hearing loss, grade of trauma on the Eshraghi scale (Eshraghi and Van de Water, 2006) and volume of tissue response. No correlations were statistically significant.

<table>
<thead>
<tr>
<th>Distance (RWM to CO)</th>
<th>Hearing Loss</th>
<th>Trauma Grade</th>
<th>Tissue Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.193</td>
<td>0.052</td>
<td>0.041</td>
</tr>
</tbody>
</table>

5.4.3 Tissue response and trauma

Using Spearman-Rho correlation and when considering all cohorts, higher grades of trauma was not associated with tissue response ($r = -0.277$, $p = 0.181$)

5.5 Additional Discussion

As with Chapter 4, these results showed that the surgeon could successfully control for cochleostomy location. This included minimal variability, as determined by a low standard error of the mean (0.02 mm, or 2% of baseline mean), and with no correlation to hearing loss or surgical trauma.

Force recordings in the present study were correlated with grade of surgical trauma but with no relationship to post-implantation hearing loss. This result is consistent with a recent study by Drouillard et al. (2017), that used a motorised arm for implantations in guinea pigs and measured force from beneath the animals’ head. No relationship between peak force and hearing loss was identified. The mean peak forces of their motorised study and peak forces in our study did not differ substantially (hard of 56 vs 42 mN for our study; soft of 26 vs 28 mN). However, the motorised study had far higher variability of over 50% of the baseline mean (23.8 mN SEM with baseline of 56 mN), compared to 11% of the baseline mean in the present study (4.6 mN SEM with baseline of 42 mN). The ability to make fine adjustments during the procedure, such as with respiration of the animal, with a manual operator may have contributed to less variability in our results. A second explanation is that touch alone can detect surgical resistance better than force recordings; this is unlikely given the results of the present study where force spikes arose nearly 1 s before the reporting of resistance.
Ideally, this interpretation should be confirmed by conducting a trial where the surgeon is given an opportunity to alter their operative technique in response to real-time force feedback. While recording paradigms did differ, a further possibility for the level of variability between studies is the use of a standardised and validated implantation technique that was performed by an experienced operator in the present study.
Chapter 6: General Discussion
Chapter 6: **General Discussion**

The preservation of residual low frequency hearing has become a recognized goal of CI surgery to optimize the performance of perceptual tasks relying on pitch resolution, such as listening in noise or to music. Unfortunately, post-implantation losses of residual low frequency hearing occur in half of all patients (Carlson et al., 2011) and this degrades many of these performance benefits. While surgical trauma and its resultant biological response are believed to be the main factors contributing to residual hearing loss, their precise role remains indeterminate, in part, because of the lack of a standardised implantation model.

The studies undertaking during the completion of this thesis were conducted to examine the different types of surgical trauma, how they relate to hearing loss and how hearing loss can be prevented. A standardised and replicable model of hearing preservation cochlear implantation surgery was first defined using a novel, whole-specimen imaging technique. Modelling demonstrated the need for accurate placement of the cochleostomy or RWM approach for minimising drilling and electrode insertion trauma, and for limiting the effects of the tissue response. Further determinants of surgical trauma included successful navigation of the hook region, use of a flexible electrode and a low frictional force insertion. Systemic glucocorticosteroids delivered prior to surgery were found to be effective in reducing the tissue response, although this was highly dependent on the level of surgical trauma, and in mitigating low frequency hearing loss, as measured by the amplitude of the response and not threshold. Surgical trauma and post-implantation hearing loss was best predicted by intraoperative CAP responses in response to a novel multi-tone stimulus when compared to CM responses and force recordings. Collectively, these results have important implications for trauma minimisation and hearing preservation strategies.

### 6.1 Determinants of surgical trauma

#### 6.1.1 Surgical technique

The identification of a reliable and clinically applicable model for hearing preservation cochlear implantation surgery was undertaken in Chapter 3. Modelling in this study identified the anteroinferior margin of the RW or adjacent otic capsule of the guinea pig to allow direct implantation into scala tympani, with a safe margin from important cochlear
structures. Cochleostomies that were positioned too anterior were likely to result in drilling or immediate electrode insertion trauma to the basilar membrane or spiral ligament, and even direct entry into scala media. Cochleostomies placed too inferior had the greatest risk of direct venous damage, which can have important consequences on hearing. Importantly, these structural relationships and findings on surgical approach replicate similar human anatomical studies (Li et al., 2007b, Atturo et al., 2014, Stidham and Roberson, 1999).

Modelling also showed that a cochleostomy placed closer to the RWM produced lower forces on initial outer wall contact. This was predicted to increase the likelihood of successful advancement, compared with cochleostomies placed further away from the RWM. This result is especially relevant given the tighter curvature of the basal turn of the guinea pig cochlea compared to humans and highlights the particular attention required of cochleostomy placements in guinea pigs. In addition, we showed that the cochleostomy can be successfully placed in a repeatable position close to the round window in the guinea pig cochlea (Chapter 4 and 5).

6.1.2 Electrode features

Inserting with stiffer electrodes resulted in much higher rates of macroscopic trauma. After controlling for surgical technique, 28% of insertions with stiffer electrodes were traumatic (in Chapter 5) compared to only 10% with a highly flexible electrode (in Chapter 4). Important features of the implant that contributed to surgical trauma were the use of a firmer silicone and a straight platinum wire (in Chapter 5). In comparison, the highly flexible electrode of Chapter 4 had a helical designed wire, as used in contemporary designed hearing preservation implants. Although rare, trauma with a flexible electrode was found to occur with all depths of insertion and mostly confined to the region of the hook, remote from the tip of the electrode. As such, trauma with a flexible electrode most likely results from a poor initial insertion trajectory and not increased insertion depth.

6.2 Determinants of the tissue response

The 4-week tissue response in Chapter 4 was shown to be directly related to the depth of insertion with a highly flexible electrode, suggesting that the extent of inflammation elicited within the cochlea grows in proportion with the depth of the electrode insertion. For example,
in control (or untreated) cochleae, the level of fibrosis, in terms of total volume and extent of scala tympani involvement, increased proportionally with the depth of insertion.

Surgical trauma was found to exert a loco-regional effect on tissue response. However, it did not cause a global increase in tissue response throughout the cochlea unless trauma was also widespread. For example, it was found in Chapter 4 that overt trauma in the hook region was associated with a robust local tissue response, but this did not dramatically affect the linear increase of tissue response through the rest of the cochlea. Furthermore, Chapter 5 confirmed that no correlation between grade of trauma and tissue response existed when depth of insertion was controlled for.

Finally, two regions where the tissue response occurred irrespective of the depth of insertion were retrograde to the cochleostomy (and towards the RWM) and anterograde (or distal) to the tip of the electrode array. Retrograde scarring that extended from the cochleostomy to the RWM and involving at least 5% of its endothelial lining arose despite the introduction of the electrode via a cochleostomy positioned 0.8 mm from the edge of the RWM. RWM scarring was found to occur with OSL fracture but also without fracture and this has been previously reported in the temporal bone of a hearing preservation implant recipient (Quesnel et al., 2015). Potential explanations include the inadvertent entry of bone dust, which has been shown to promote an extensive inflammatory response (McElveen et al., 1995), or intracochlear blood that forms scaffolding for the tissue response (Kel et al., 2013). For the experimental procedures in Chapter 4 and 5, flushing of visible bone dust outside of the cochlea was performed. However, it is highly feasible that some bone dust inadvertently entered the cochlea of animals with RWM scarring. An anteroinferior plane was used in these experimental procedures as this provided a safe distance from all important structures identified in Chapter 3’s modelling of the guinea pig cochlea. This included all major veins and with a safety margin of atleast 0.5 mm. But it is possible that smaller vessels, unaccounted for by TSLIM modelling, were damaged during placement of the cochleostomy and this may have resulted in the intracochlear entry of blood. Extension of the tissue response beyond the tip of the electrode of at least 30 degrees (or approximately 1 mm) occurred in several animals. Importantly, this did not extend beyond the first turn and is unlikely to be a contributor of post-implantation hearing loss.

*Chapter 6: General discussion*
6.3 Post-implantation hearing loss

6.3.1 Tissue response and post-implantation hearing loss

Overall, the level of tissue response was not correlated with post-implantation hearing loss (Chapter 5). However, specific types of tissue responses were found to be directly associated with or deemed to be at risk of causing hearing loss. RWM scarring in Chapter 4 occurred with low frequency hearing loss of more than 15dB. This result has been previously demonstrated experimentally through adhesives applied on the middle ear surface (Nageris et al., 2012). The proposed mechanism of hearing loss in this setting is from an increase in cochlear impedance, such that sound waves face high resistance as they travel toward the scala vestibuli and only a portion of acoustic energy will actually reach the cochlear duct (Nageris et al., 2012). However, the attribution of hearing loss to RWM scarring is tempered by the occurrence of hook region fracture in half of these animals.

Modelling of the guinea pig cochlea in Chapter 3 revealed that a robust tissue response may endanger some intracochlear structures. These structures included the cochlear aqueduct and ductus reuniens that were remote from most surgical approaches but at risk from a robust tissue response near the inferior margin of the RWM. Occlusion of the ductus reuniens from trauma or tissue response is one proposed mechanism for the development of endolymphatic hydrops that has been observed in implant recipients (Quesnel et al., 2015, Richard et al., 2012, Handzel et al., 2006). Results from this Department recently showed endolymphatic hydrops, detected on micro-CT, to be prevalent in the weeks following cochlear implantation in experimental surgery (Smeds et al., 2015). Endolymphatic hydrops occurs when the volume of endolymph inside the scala media increases, causing the pliant basilar membrane and Reissner’s membrane to bow (Salt et al., 2012). While TSLIM in the present studies enabled visualisation of these membranes, the effects of dehydration and shrinkage from processing steps on the volumes of endolymph and perilymph have not yet been established (Buytaert et al., 2013).

6.3.2 Trauma and post-implantation hearing loss

From Chapter 5, post-implantation hearing loss was not correlated with a higher grade of surgical trauma. And while OSL fractures of the hook region tended to have worse hearing in Chapter 4, this was confounded by substantial RWM scarring. A consistent finding of this
thesis was that insertions traditionally considered ‘atraumatic’, and in the absence of gross trauma, could still result in significant hearing loss. This result is supported by Eshraghi et al. (2005), whereby rats implanted with minimal trauma were found to have substantial hearing losses. Ultimately, these findings led Eshraghi and colleague(s) to revise their trauma scale to include ‘molecular’ or ‘microscopic’ trauma as the lowest level (Eshraghi and Van de Water, 2006). However, our results demonstrate that this scale remains arbitrary and without meaningful correlations to hearing loss.

Postulated causes of ‘molecular’ or ‘microscopic’ trauma include molecular injury to the organ of Corti arising from inflammation (Eshraghi et al., 2005, Eshraghi and Van de Water, 2006) or from fluid displacement within scala tympani (Harris et al., 2017). Another proposed mechanism of hearing loss in the absence of gross trauma is from micromechanical changes occurring from contact between the electrode and basilar membrane. Campbell et al. (2016) found sudden recovery of electrophysiology occurred with a change in position of an electrode that usually confirms to the lateral wall and basilar membrane (Verberne et al., 2016). They hypothesised that contact and elevation of the basilar membrane altered the cochlear mechanics and that when the position of the electrode was shifted away from the cochlear partition, the basilar membrane movement was suddenly restored, along with hearing (Campbell et al., 2016). A simulation with human cochlea and basilar membrane fixation has shown similar alterations to hearing (Kiefer et al., 2006). This is a highly plausible explanation for hearing loss in the present thesis, since the physical contact of the basilar membrane with contemporary human electrode (Verberne et al., 2016, Skarzynski and Podskarbi-Fayette, 2010, Mukherjee et al., 2012) was successfully replicated in Chapter 4.

6.4 Role of systemic glucocorticosteroids in cochlear implantation

6.4.1 Steroids reduce tissue response in atraumatic surgery

The efficacy of systemic steroids to reduce the total volume of fibrosis was affected by the level of trauma. For example, systemic steroids delivered before insertion with an atraumatic, highly flexible electrode (in Chapter 4) reduced the total volume of tissue response, but this effect was greatly diminished for traumatic insertions. Stiffer electrodes (in Chapter 5) that caused far higher rates of trauma (28% vs 10% in Chapter 4) had no steroid effect on the tissue response volume. In Chapter 4, loco-regional steroid effects were noted in the

Chapter 6: General discussion
presence of specific pathologies. For example, steroids reduced the proportion of scala tympani occupied by fibrosis in apical regions of the cochlea, where contact between the basilar membrane and lateral wall was the commonest type of injury. Similarly, steroids reduced fibrosis around the hook region and in proximity to the cochleostomy, but only in the absence of severe cochlear trauma.

The results of this study have provided some useful insights into the likely mechanisms of action of systemic steroids. Highly traumatic insertions, such as with a fracture of the OSL, result in the entry of blood and subsequent clot formation in to scala tympani. Previous research by our Department has shown that the entry of blood after implantation in guinea pigs leads to an ingress of fibroblasts into the clot (Kel et al., 2013). These fibroblasts migrate to affect fibrosis, which occurs towards the end of the first week after surgery (Kel et al., 2013). Despite the change of cell types, the volume of the tissue response does not change significantly (in the absence of steroids) between 24 hours and 3 months after surgery (Smeds et al., 2015). The results of this thesis suggest that a single dose of systemic steroids is unlikely to affect this ingress of fibroblasts. However, a single preoperative dose of systemic steroid could directly reduce clot formation by potentially stabilizing the blood-labyrinthine barrier or by decreasing the exudation of protein and clotting factors into scala tympani (Boumpas et al., 1993). These considerations may explain why the tissue response was reduced deep in the cochlea (in Chapter 4), away from regions affected by direct trauma to the scalar walls, and where fibrin would likely have entered scala tympani via an increase in vascular permeability. Additionally, this interpretation may explain why fibrosis remained exuberant when traumatic insertions had occurred and despite the administration of steroids. This explanation might also account for the small (or absent) effect on fibrosis with local steroid administration in some studies (Chang et al., 2009, James et al., 2008, Stathopoulos et al., 2014, Huang et al., 2007), since locally administered steroids have a lower cochlear tissue profile and less effect on inflammatory cells (Bird et al., 2011).

### 6.4.2 Steroids improve the magnitude of low frequency hearing

In Chapter 5, systemic steroids administered before surgery were shown to be effective in maintaining CAP amplitude losses, that otherwise fell in response to suprathreshold acoustic stimulation. This result was most pronounced in response to low-mid frequency sounds. Interestingly, no such steroid-mediated effect was found on hearing thresholds to any
frequency of sound, in both Chapter’s 4 and 5. This result is supported by Ye et al. (2007) that showed topical steroid administration improved short-term hearing amplitudes but not threshold in guinea pigs following implantation. Reiss et al. (2015) recently showed that a post-implantation reduction in CAP amplitude growth for low frequency sounds correlated with losses in the pre- and post-synaptic terminals of hair and spiral ganglion cells. Unfortunately, TSLIM in the present thesis did not permit the counting of synaptic ribbons. However, these results do lead to the possibility that synaptopathy, if present, may be amenable to steroid treatment and this is another area of future investigations arising from this thesis (6.7 Directions of Future Studies).

6.4.3 Steroids have no effect on high frequency hearing

No steroid-mediated effect was found on CAP amplitude or threshold in response to high-frequency stimuli across experiments in this thesis. Recently, deteriorations in high frequency hearing after implantation have been attributed to the collapse of smaller or branched capillaries of the stria vascularis, located in the upper portion of the spiral ligament (Reiss et al., 2015, Tanaka et al., 2014). Collapse of stria vascularis and adjacent vessels occurring after trauma is believed to result in deteriorations in the endocochlear potential that is necessary for hair cell transduction of sound (Reiss et al., 2015, Tanaka et al., 2014). A similar finding on stria vascularis and deteriorations in the endocochlear potential has been found with other cochlear insults, like noise exposure, ototoxic antibiotics and lipopolysaccharide, a bacterial endotoxin (Li et al., 2011, Koo et al., 2015). While hearing preservation CI surgery is mainly concerned with residual low frequency hearing, effects on stria vascularis function and endocochlear potential may have broader effects on hearing and/or implant performance.

The lateral wall is often deemed as the safest point of initial contact for the tip of a straight electrode because of a lower risk of trauma to the organ of Corti and related structures (Shapira 2011). As such, the outer lateral wall was used as the safest initial contact for modelling purposes in Chapter 3. This guided the surgical approach used for Chapter’s 4 and 5, where outer lateral wall contact was achieved with nearly all insertions. According to the principles of least resistance, a straight electrode is directed toward the most lateral point of the scala when contacting the lateral wall (Roland, 2005). Since scalar wall curvature becomes steeper further along the cochlea and away from the RWM (Verberne et al., 2016),
misguided and/or deeply inserted electrodes may be predisposed to tearing or contacting the tissues of the spiral ligament complex or stria vascularis. Further experiments exploring the specific types of implantations and trauma that result in stria vascularis changes are warranted, especially if these changes do affect residual hearing or implant performance (Reiss et al., 2015, Tanaka et al., 2014).

6.5 Intraoperative monitoring

6.5.1 Electrocochleography

The efficacy of two intraoperative recording techniques, ECochG and force, for detecting hearing loss and surgical trauma was investigated in Chapter 5. The principal finding was that losses in the intraoperative CAP response correlated with higher grades of trauma and greater post-implantation hearing loss. Clinical studies have shown that intraoperative ECochG losses may be associated with incomplete hearing preservation (Campbell et al., 2016, Dalbert et al., 2016, Mandala et al., 2012, Radeloff et al., 2012). However, these studies have been largely in the presence of traumatic insertions, including scalar translocation (Dalbert et al., 2016, O’Connell et al., 2017), kinking of the electrode (Radeloff et al., 2012) and from a fast insertion speed (Mandala et al., 2012).

This is the first intraoperative ECochG study to correlate electrode path and grade of trauma with post-implantation hearing. A notable finding of Chapter 5 was that an atraumatic insertion (without gross morphological damage) could result in a decline in the intraoperative CAP (at the time of surgery) and result in a long-term hearing loss. It is highly plausible that intraoperative CAP recordings detect molecular and micromechanical causes of hearing loss, in addition to more traumatic causes, and this may explain the strength of the correlation we found with post-implantation hearing loss. This result is further supported in acute studies with gerbils, whereby intraoperative CM and CAP amplitude losses occurred with ‘scrapes’ or minor contact to the OSL or basilar membrane (DeMason et al., 2012, Choudhury et al., 2014).

A further finding of this study was that no such correlation with hearing loss and trauma was found for any of the intraoperative CM responses. The CM recorded from an intracochlear electrode has been found to be reliably recorded (Campbell et al., 2015), even in some
individuals with no measurable audiometric thresholds (Choudhury et al., 2012). So far, it has been shown to be a more useful predictor of post-implantation hearing loss than the CAP (Campbell et al., 2016). This contrasts with Chapter 5 findings that the CAP was the better predictor of post-operative hearing. This finding is suspected to arise from the use of a novel multi-tone acoustic stimulus. Previous clinical and animal studies have used a pure-tone acoustic stimulus from a frequency consistent with the patients’ (or animals’) residual hearing (Campbell et al., 2015, Campbell et al., 2016, Choudhury et al., 2014, Choudhury et al., 2011). Conversely, the multi-tone stimulus used in Chapter 5 originated from 3 different turns of the guinea pig cochlea: basal, first and second turns. As such, any reduction in CAP amplitude with the multi-tone stimulus likely represented a decline in cochlear function from any of the three sites generating the summed acoustic response. Consequently, intraoperative CAP responses were found to be more sensitive to cochlear trauma than localised CM responses that reflect hair cell activity in the vicinity of the electrode only (Campbell et al., 2017).

6.5.2 Force

In vivo uni-axis force experiments in Chapter 5 were found to be complex, multi-peak traces that were associated with higher grades of trauma, but not with hearing loss. In addition, a sudden rise in force (or secondary rise, when present) usually preceded the reporting of resistance. This suggests that monitoring for a rising intracochlear force can detect surgical resistance earlier than by touch alone. However, the rise in force usually occurred less than a second before the reporting of resistance. This may limit the application of this particular intraoperative monitoring modality given the relatively slow human reaction times (see 6.6.4 Implications of force recordings). By comparison, a decrease in the intraoperative CAP amplitude preceded the reporting of resistance by 6 seconds.

6.6 Implications for the current work

6.6.1 The guinea pig has structural similarities to the human cochlea

The guinea pig cochlea has been extensively used by our Department and other research groups, but with various implantation techniques and results on hearing. Using a novel whole-specimen imaging technique, structural relationships of the guinea pig were found to
be consistent with human three-dimensional anatomical studies (Li et al., 2007b, Atturo et al., 2014, Stidham and Roberson, 1999). In addition, the safety of an anteroinferior placed cochleostomy for minimising insertion trauma and variability in experimental guinea pigs was modelled (Chapter 3) and validated (Chapter’s 4 and 5) in this thesis. This finding also concurs with results of human anatomical studies for the safety of this plane (Guo et al., 2016, Li et al., 2007b). Experiments with guinea pigs that control for the surgical approach are likely to provide useful insights into the aetiology and the development of therapies directed at post-implantation hearing loss. This includes investigations on electrode design and surgical technique, along with drug delivery strategies.

An important consideration of findings on the guinea pig cochlea is that the structural relationships are approximately half the magnitude of the human cochlea, owing to the smaller dimensions of the hook region and lower basal turn. Furthermore, there is a far greater coiling of the guinea pig cochlea that results in a much more rapid transition from the hook to the spiral region. Researchers must take extra care when placing the cochleostomy or performing RWM entry in the guinea pig since this can result in a much larger discrepancy of electrode trajectory when extrapolated onto the tightly coiled outer wall.

Experimental electrodes implanted in animals should be validated in vivo to ensure they replicate the behaviour of contemporary electrodes (Skarzynski and Podskarbi-Fayette, 2010, Lenarz et al., 2006, Mukherjee et al., 2012). For example, the guinea pig electrode designed in Chapter 4 achieved consistent physical contact (with potential trauma at the microscopic level) of the basilar membrane and lateral wall. But implanting guinea pigs with human implants may not be applicable because of the proportional differences highlighted. For example, straight human implants are designed for first contact with the outer wall of 110 to 180 degrees (Breinbauer and Praetorius, 2015, Escude et al., 2006), which is a similar range to the surgical approaches identified in Chapter 3. However, this range of angular depths translates to a linear distance of 8 to 12 mm in humans compared with just 3 to 4 mm for ideal insertion vectors in the guinea pig.

6.6.2 Standardised and replicable modelling techniques

Standardised and replicable planes of view for analysing cochleae were presented in Chapter 3. These planes (axial and radial) allow angular measurements to be consistently performed.
and compared between researchers. Furthermore, the methods in Chapter 3 for the angular descriptions of the ideal insertion vector could be applied to human cochleae, particularly since the ideal insertion vector has not been analysed experimentally for the preservation of hearing or for structural integrity.

The axial plane, defined in Chapter 3, closely corresponds to the ‘Cochlear View’ (Xu et al., 2000) that is in clinical use for documenting the results of cochlear implantation and in evaluating the depth of insertion and electrode position. The radial plane corresponds approximately to a cross-section of the basal turn (Roland, 2005) and directly relates to important neural and soft tissue structures. The radial plane is poorly defined with current imaging techniques of implant candidates because of poor resolution and artefact. Furthermore, histological preparation techniques will section in the mid or para-modiolar orientation with large intervals that result in a loss of detail (Verberne et al., 2016). Hence, whole-specimen imaging techniques like TSLIM, used in the present study, and micro-CT that allow spatial orientation and digital reslicing in any desired plane in high resolution are optimally suited for these types of experiments. Additional steps of stitching high-resolution images, as encountered in Chapter 3, and segmentation and reconstruction with software may be also required. An important limitation of Chapter 3 was that modelling was performed exclusively with straight insertion vectors since both RW and cochleostomy approaches were analysed and only straight electrodes can be reliably performed on both (Souter et al., 2011). The validation of an ideal insertion vector for precurved perimodiolar electrode arrays should ideally be performed with a study of similar methodology.

6.6.3 Residual low frequency hearing

In Chapter 5, sustained shifts in CAP amplitude of low-mid frequencies were identified after implanting with a hard electrode, despite a partial recovery in threshold. An important implication for EAS patients is that effective residual low frequency hearing loss is not adequately assessed by classical audiometry, which assess thresholds only. Synaptic damage (or synaptopathy) is increasingly recognised as a consequence of traumatic noise exposure (Lin et al., 2011, Kujawa and Liberman, 2009) despite the recovery of cochlear thresholds. Recently, Reiss et al. (2015) presented data that suggested synaptopathy may also occur after experimental cochlear implantation and this is supported by our results in Chapter 5. Threshold recording configurations in Chapter 4 were successfully modified for Chapter 5.
to include peak-to-peak amplitude calibrations to derive amplitude shifts. Similar techniques may be applied to implant recipients, and even in some individuals with no measurable audiometric thresholds (Choudhury et al., 2012, Campbell et al., 2016), to determine if synaptopathy is present after cochlear implantation.

CAP amplitude losses with hard electrodes in Chapter 5 were offset by systemically administered steroids. This has the tantalising possibility of improving hearing outcomes for patients undergoing hearing preservation cochlear implantation surgery. In consideration of the findings of Reiss et al. (2015), the results of Chapter 5 suggest that steroids administered prior to surgery may prevent denervation and synaptic damage. This interpretation is supported by previous studies that have shown in experimental cochlear implantation, steroids act directly on inner hair cells through anti-inflammatory, anti-apoptotic and antioxidant pathways (Dinh et al., 2008, Haake et al., 2009, Van De Water et al., 2010a, van de Water et al., 2010b). Ideally, the positive electrophysiological findings of steroid application in the present study would be supported by preservation of presynaptic ribbons and postsynaptic receptor counts (Reiss et al., 2015). Unfortunately, TSLIM did not allow us to perform this and the processing steps of dehydration and clearing precluded any post-imaging analysis. Hence, a useful adjunct to the current experimental design would be a histological analysis of implanted cochleae of control and systemic steroid animals for counts of hair cells and synaptic ribbons.

6.6.4 Intraoperative monitoring

Recording paradigms for intraoperative electrocochleography

Losses in the intraoperative CAP response to a simple multi-tone stimulus correlated with higher grades of trauma and greater post-implantation hearing loss, however, no such correlation was found for any of the intraoperative CM responses. Previous clinical studies have used a pure-tone as the acoustic stimulus. In these studies, the CM tends to grow as the electrode is advanced into the cochlea and approaches the cochlear site generating the response (Bester et al., 2017). Decline in the CM response recorded intraoperatively from the electrode are likely to represent hair cell activity in the vicinity of the electrode only (Campbell et al., 2017). The observations in Chapter 5 raise the possibility that the CM may not be the ideal cochlear potential to record and that a global ECochG response, such as a multi-tone CAP, may offer a more robust feedback mechanism for improving hearing.

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preservation rates. However, CAPs are usually not seen with CI candidates (Campbell et al., 2016), hence, it may not be practical to use CAP monitoring in the majority of surgeries.

**Implications of force recordings**

The cumulative force of insertion, measured from the uni-axis sensor in Chapter 5, was correlated with higher grade of surgical trauma. Additionally, a peak in force nearly always preceded the reporting of resistance by the surgeon. This means that monitoring for a rising intracochlear force can detect surgical resistance earlier than by touch alone. However, the force peak preceded resistance by less than 1 s for most insertions and the latency of the human response is one factor that may limit how effective this feedback is for minimising trauma in the clinical setting.

Importantly, this result does highlight the importance of speed control when nearing a full insertion depth. In this study, where the insertion speed was 15 mm/min, a 1 s latency in detecting resistance would mean the electrode travelled approximately 0.25 mm further from the force change than by the time the surgeon stopped the insertion. The electrode would have advanced less, and likely caused less trauma, if the speed of insertion was slowed, and vice-versa if increased. Considering that typical insertions are much faster, range of 42 – 165 mm/min (Kontorinis et al., 2011a), this result has important implications for minimising trauma towards the end of the insertion, where residual hearing is believed to be often lost (Campbell et al., 2017).

Two important findings arose from the force recordings in to the scala tympani model in Chapter 5. Firstly, the behaviour of the electrode insertion in to the scala tympani model changed depending on the stiffness of the implant. From reviewing video footage, hard electrodes required large insertion forces to overcome the point of impingement from first contact with the wall of the scala tympani model (Zhang, 2010). The soft silicone electrodes appeared to have less impingement in the scala tympani model than hard electrodes. A second finding was that lower forces were found with the experimental procedure compared to the scala tympani model, especially for hard electrodes (mean peak of 42 mN for the experimental procedure vs 102 mN for the model). One possible explanation is the use of a sensor built in to a hand-held insertion tool for experimental procedures compared to the simple, vertical setup used with the scala tympani model. Frictional forces were reduced

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through the user of carbon fibre tubing (with low frictional coefficient) and the lubricant Dextran. Furthermore, the hand-held insertion tool was found by Miroir et al. (2012) to be comparable to a conventional 6-axis sensor placed beneath the specimen.

A more probable explanation for the differences between in vivo and ST model results is that frictional forces arising from live tissue differed substantially to the model. And more specifically, that the lateral wall soft tissues have far lower frictional forces than the rigid epoxy model. Additionally, these results demonstrate that the insertion profile of an electrode, when studied ex vivo, may differ to their actual behaviour in vivo. Development and evaluation of cochlear implant designs are typically performed in acrylic or epoxy models (Adunka et al., 2004, Schurzig et al., 2010). This is usually followed by cadaveric temporal bones that are then analysed via conventional bone histology (Briggs et al., 2006, Souter et al., 2011, Adunka et al., 2004) or microdissection (Roland and Wright, 2006). Cadaveric temporal bones may undergo freezing, preserving or be placed in fixative agents after an unspecified period of time, all of which may alter the nature of intracochlear soft tissues. Future studies should consolidate these differences between preparation techniques of cadaveric temporal bones. Finally, studies of force and insertion profile performed on scala tympani models should be approached with caution and, ideally, be accompanied by studies performed on temporal bones and later, in vivo.

6.6.5 Steroid effect on tissue response

Systemic steroids delivered before the insertion of an atraumatic, highly flexible electrode reduced the total volume of tissue response (in Chapter 4). Furthermore, steroids reduced fibrosis with deeper insertions and where contact between the electrode and basilar membrane frequently occurred. Specific tissue responses were also found to be associated with hearing loss, including proximal RWM scarring, and this result suggests a potential role for steroid therapy to prevent delayed hearing loss. A further implication of reducing tissue responses is the effects on the electrode-neural interface. Fibrosis in proximity to the electrode has been shown to increase electrode impedance, degrade signal fidelity (especially from telemetry through thick skin) and increase the voltage required to excite neural structures to threshold (Chikar et al., 2012). Ultimately, this leads to reduced battery life and poor performance from degraded signal fidelity (Gu et al., 2016). Hence, systemic steroid therapy and its actions on tissue response may have far reaching consequences on
performance, battery life and future proofing (or preserving) the ear for future therapies.

6.7 Directions of Future Studies

6.7.1 Defining the ideal cochleostomy and round window approach

The unpredictability in angular depths and trauma identified among implant recipients (Finley et al., 2008, Breinbauer and Praetorius, 2015, Boyer et al., 2015) may be, in part, consolidated by exploring variations in cochleostomy placement or round window entry. Surveys of implant surgeons have confirmed numerous inconsistencies in surgical approach and cochleostomy technique, including a recent study that showed 8-10% of surgeons surveyed would elect for a traumatic superior location (Iseli et al., 2014). Results of the three-dimensional study in Chapter 3 suggest that the guinea pig would be a useful model for determining this.

One component of the cochleostomy that is still yet to be determined is how far it should be placed from the RWM. Cochleostomies have the theoretical advantages of avoiding some of the curved hook region and with a better alignment to the centreline axis (Meshik et al., 2010, Shapira et al., 2011). From results of Chapter 3, cochleostomies placed further from the RWM achieved a deeper insertion depth and navigated less of the hook region and theoretically, with reduced risk of hook region trauma. However, a shallow insertion arising from a more proximally located cochleostomy was found to contact the outer wall where there is a gentler curvature of the cochlea, thus causing a lower initial outer wall force. Another consideration is the accumulative force of the insertion, as opposed to the instantaneous force of the first contact. For example, a shallow insertion onto the outer wall may have longer travel along the outer wall than a deep insertion, resulting in higher overall frictional forces that must be overcome to advance the electrode. A well-designed study that factors the anatomical relationships and plane of insertion in the guinea pig may help address this outstanding research question.

6.7.2 Unexplored causes of surgical trauma

The results of Chapter’s 4 and 5 highlights that post-implantation hearing loss can occur in the absence of gross morphological trauma. Since these studies successfully replicated the
physical contact of the electrode on the basilar membrane (and lateral wall), one proposed area of future research is the effect of basilar membrane contact or fixation on long-term hearing loss. Verberne et al. (2016) showed that a straight electrode makes contact with the basilar membrane with increasing depths of insertion owing to the shape of the scala tympani as it moves toward the second turn of the cochlea. Furthermore, Campbell et al. (2016) found that recoverable losses in the intraoperative ECochG occurred towards the end of the insertion, which is presumed to occur from trauma or tenting of the basilar membrane. However, these are not conclusive findings. Further experiments are required to determine what effect basilar membrane contact has on membrane function (both instantaneous and later) and if this is in fact detrimental to long-term hearing. The types of basilar membrane contact should be further defined to include fixation and non-fixation (Kiefer et al., 2006).

6.7.3 Investigating the biological response

In Chapter 3, the location of the ductus reuniens relative to important structures was identified and discussed, including the potential effects of occlusion and the development of endolymphatic hydrops. Previously, endolymphatic hydrops was identified in guinea pig cochleae following implantations performed by our Department (Smeds et al., 2015). Cochleae were analysed through the use of micro-CT and hydrops was found to be prevalent despite minimal evidence of electrode insertion trauma (Smeds et al., 2015). Endolymphatic hydrops has been shown to be present with a sensorineural hearing loss, such as Meniere’s Syndrome (Merchant et al., 2005). It is believed that the bowing of the basilar membrane in hydrops (from an increased scala media volume) causes conformational changes on the stereocilia of sensory hair cells away from their optimal position, thereby reducing their sensitivity to sound (Durrant and Dallos, 1974). Translating these findings to post-implantation hearing loss is entirely speculative and it remains unclear how ductus reuniens occlusion, or endolymphatic hydrops itself, develop or what their actual contributions to hearing loss are.

Endolymphatic hydrops was unable to be analysed with TSLIM in the present study because of the uncertain effects of dehydration from tissue processing and compartment shrinkage. Because soft tissues are not present, it is not possible to individually segment the scalae from TSLIM images (Buytaert et al., 2013). Theoretically, one post-processing solution is to use the autosegmentation of AMIRA to fill the empty spaces occupied by fluid or soft tissue.
A second effect of the biological response that warrants further investigation is synaptopathy as a delayed cause of post-implantation hearing loss. ‘Hidden hearing loss’ is a rapidly emerging form of hearing disability that is characterised by perceptual difficulties but with a normal audiogram (Plack et al., 2014). A landmark study by Kujawa et al. (2009) showed that mice exposed to noise experienced a selective loss of synapses despite a full recovery of sensitivity to quiet sounds. Furthermore, the amplitude of the ABR was normal at low sound levels but reduced at medium to high sound levels (Kujawa and Liberman, 2009). Similarly, Reiss et al. (2015) recently showed that a post-implantation reduction in CAP amplitude growth was correlated with losses in the pre- and post-synaptic terminals of hair and spiral ganglion cells. This result is supported by Chapter 5 findings of predominant amplitude shifts with some recovery of threshold after implanting with a hard electrode. Unfortunately, anatomical correlations with synaptic changes were unable to be made with the use TSLIM. Hence, further research is needed to better define this potential cause of hearing loss.

Finally, several techniques to minimise direct trauma warrant additional exploration. These solutions, mostly in the early stages of development and validation, include robotic drills for minimising damage to the cochlear endosteum (Coulson et al., 2008) image-guided and/or motorised insertions (Zhang et al., 2010, Matsumoto et al., 2009, Mamelle et al., 2017), as well steerable electrode arrays (Zhang et al., 2010).

### 6.7.4 Optimising drug delivery

From the results of Chapter 4 and 5, the proposed mechanism of action for a single preoperative dose of systemic steroids to reduce tissue response is via stabilisation of the blood-labyrinthine barrier or by decreasing the exudation of protein and clotting factors into scala tympani (Boumpas et al., 1993). Important limitations of systemic administration highlighted by this study are that it likely has little effect on the entry of blood in to scala tympani after implantation nor the ingress of fibroblasts in to the clot that affects fibrosis. Hence, two areas of future research are clot reduction and preventing the ingress of
fibroblasts. Clot prevention may occur by altering the properties of platelets, coagulation factors or fibrin (Kumar et al., 2013). Alternatively, it may arise from clot dissolution, termed fibrinolysis. These therapies are likely to be administered locally, as opposed to systemic therapy with steroids, because of the potential for side effects.

A further area of potential research highlighted by this thesis is reducing the ingress of fibroblasts. For example, steroid-eluting cochlear implants electrodes have been shown to reduce fibrosis (Wilk et al., 2016, Wrzeszcz et al., 2014). However, their action is under very different circumstances to that studied in this thesis since no drug is present in the cochlea at the time of electrode insertion and so there can be no drug effect upon the immediate inflammatory response and clot formation. It is instead hypothesised that slow steroid elution in to scala tympani results in a slowing of the migration of fibroblasts into the clot, which instead undergoes fibrinolysis. If this interpretation is true, then steroid administration via systemic and drug-eluting means may have complimentary actions for reducing the tissue response. At this stage, the steroid releasing profile and potential effects on cochlear soft tissue and bone are still being determined for drug-eluting electrodes (Niedermeier et al., 2012).

The steroid effect on CAP amplitudes identified in Chapter 5 raises the possibility that synaptopathy, if present, may be amenable to steroid treatment. Previous studies have shown that in experimental cochlear implantation, steroids act directly on inner hair cells through anti-inflammatory, anti-apoptotic and antioxidant pathways (Dinh et al., 2008, Haake et al., 2009, van de Water et al., 2010b). Hence, a potential further area of research is the characterisation of synaptopathy after implantation and steroid and with the use of inflammatory markers. Another area of research worth exploring is the timing of synaptopathy, since this may guide the optimal delivery of steroids to preserve residual low frequency hearing. Finally, further clinical studies are needed to prove whether these benefits can be realized in actual cochlear implant recipients.

6.7.5 Hearing preservation manoeuvres

This thesis analysed two intraoperative modalities, force and ECochG. ECochG recordings and specifically CAP responses, were shown to provide a continuous real-time recording that correlated with surgical trauma and predicted long-term hearing loss. Intraoperative force...
also correlated with physiological damage but with limitations on hearing loss and a reliance on a small reaction time for clinical application. Since intraoperative recordings in the present thesis were strictly observational, manoeuvres were not performed. However, these findings should be confirmed by conducting a trial where the surgeon is given an opportunity to alter their operative technique in response to real-time feedback on the force or CAP recordings. One modification of recordings in Chapter 5 that is needed for clinical testing of manoeuvres is video recording. Synchronisation of intraoperative monitoring and surgical technique is crucial for identifying causative and preventing manoeuvres of intraoperative losses and hearing preservation.

Intraoperative ECochG has the potential to modify several areas of the implantation technique, including when to discontinue the insertion and whether withdrawal or rotation of the electrode should be performed. In time, intraoperative ECochG may also allow more broader modifications of the surgical technique, such as optimising the insertion speed (Kontorinis et al., 2011a) and fascial plug placement (Campbell et al., 2016).

A further area of research identified by this study is whether the multi-tone stimulus in Chapter 5 can be successfully applied in the clinical setting. Changes in the CAP amplitude in response to the multi-tone stimulus had a far stronger relationship with trauma and hearing loss than any of the CM responses. This is believed to result from a broader representation of intracochlear events as opposed to only localised changes that are reflected by the CM response. Further studies should confirm whether a multi-tone stimulus has the same relationships as in the clinical setting. In practice, however, monitoring of the CAP may be challenging, since in some patients, the CAP cannot be identified at all (Campbell et al., 2015). But in the event both CAP and CM are available, studies should determine which is the preferred method.

6.8 Conclusion to Thesis

In this thesis, we were able to validate the guinea pig for cochlear implantation surgery and to demonstrate structural relationships relevant for modelling hearing preservation surgery in humans. Using this cochlear implantation model, we were able to show that surgical trauma was influenced by: accurate placement of the cochleostomy; and, electrode flexibility. The efficacy of systemic steroids on the tissue response was found to be highly dependent on the

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level of surgical trauma. Systemic steroids were found to mitigate low frequency hearing loss, as measured by the amplitude of the response but not hearing threshold. Surgical trauma and post-implantation hearing loss was best predicted by intraoperative CAP responses, compared to CM responses and force recordings.

These insights, developed from novel cochlear modelling and in vivo techniques, have provided a unique description of surgical trauma during cochlear implantation surgery. Furthermore, these results suggest a role for steroids in reducing synaptopathy and tissue response, and for electrocochleography as an intraoperative recording paradigm to reduce trauma and improve post-implantation hearing loss. These results will help inform the cochlear implant community of potential techniques to improve hearing preservation surgery.
Chapter 7: Appendix
Chapter 7: Appendix

7.1 Publications and conference presentations arising from this work

7.1.1 Publications included in this thesis


7.1.2 Co-authored publications submitted during this thesis


7.1.3 Presentations arising from this work


Chapter 8: References
Chapter 8: References


*Chapter 8: References*


Chapter 8: References


FITZGERALD, M. B., SAGI, E., JACKSON, M., SHAPIRO, W. H., ROLAND, J. T., JR.,


MAMELLE, E., KECHAI, N. E., GRANGER, B., STERKERS, O., BOCHOT, A.,


Chapter 8: References

Rhinol Laryngol, 110, 883-91.


Chapter 8: References
Chapter 8: References


SPOENDLIN, H. 1966. The organization of the cochlear receptor. *Fortschr Hals Nasen*
Chapter 8: References

Ohrenheilkd, 13, 1-227.


WILK, M., HESSLER, R., MUGRIDGE, K., JOLLY, C., FEHR, M., LENARZ, T. & SCHEPER, V. 2016. Impedance Changes and Fibrous Tissue Growth after Cochlear


Chapter 8: References
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Lo, Jonathon

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