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Relations between cochlear histopathology and hearing loss in experimental cochlear implantation

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Abstract

This study reviews the cochlear histology from four hearing preservation cochlear implantation experiments conducted on 73 guinea pigs from our institution, and relates histopathological findings to residual hearing. All guinea pigs had normal hearing prior to surgery and underwent cochlear implantation via a cochleostomy with a silastic-platinum dummy electrode. Pure tone auditory brainstem response (ABR) thresholds from 2 to 32 kHz were recorded prior to surgery, and at one and four weeks postoperatively. The cochleae were then fixed in paraformaldehyde, decalcified, paraffin embedded, and mid-modiolar sections were prepared. The treatment groups were as follows: 1) Systemic dexamethasone, 0.2 mg/kg administered 1 h before implantation, 2) Local dexamethasone, 2% applied topically to the round window for 30 min prior to cochlear implantation, 3) Local n-acetyl cysteine, 200 µg applied topically to the round window for 30 min prior to implantation, 4) inoculation to keyhole-limpet hemocyanin (KLH) prior to implantation, and 5) untreated controls. There was a significant correlation between the extent of the tissue

reaction in the cochlea and the presence of foreign body giant cells (FBGCs), new bone formation and injury to the osseous spiral lamina (OSL). The extent of the tissue response, as a percentage of the area of the scala tympani, limited the best hearing that was observed four weeks after cochlear implantation. Poorer hearing at four weeks correlated with a more extensive tissue response, lower outer hair cell (OHC) counts and OSL injury in the basal turn. Progressive hearing loss was also correlated with the extent of tissue response. Hearing at 2 kHz, which corresponds to the region of the second cochlear turn, did not correspond with loco-regional inner hair cell (IHC), OHC or SGC counts. We conclude that cochlear injury is associated with poorer hearing early after implantation. The tissue response is related to indices of cochlear inflammation and injury. An extensive tissue response limits hearing at four weeks, and correlates with progressive hearing loss. These latter effects may be due to inflammation, but would also be consistent with interference of cochlear mechanics.

1. Introduction

Delayed hearing loss following cochlear implantation remains a problem for hearing preservation surgery. Although hearing may be preserved immediately after surgery in a significant proportion of patients undergoing implantation, it has been reported that approximately a third of recipients lose their residual hearing slowly over the next few months (Barbara et al., 2003; Gstoettner et al., 2006; Woodson et al., 2010). The reasons for this are not well understood, but it has been proposed that the tissue response to implantation may be implicated. Studies of human temporal bones have demonstrated that the electrode is surrounded by a fibrous sheath (Nadol et al., 2001). It has been proposed that this tissue response⁶ may adversely affect residual hearing in several ways. The fibrous tissue could dampen cochlear mechanics if it were to involve the basilar membrane at the site of implantation in the basal turn, where it has been predicted that high frequency hearing loss will result (Choi and Oghalai, 2005; Kiefer et al., 2006). Whereas fibrosis occupying a significant proportion of the scala tympani (ST) is predicted to cause a low frequency hearing loss, according to the modelling of Choi and Oghalai (2005). Alternatively, it has been proposed that biological activity within the tissue response may be toxic to the

organ of Corti (Eshraghi et al., 2005). The data presented here provide some insights into how residual hearing may change over time after cochlear implantation.

Of the small numbers of human temporal bones available, most involve patients that have had little, if any, residual hearing and have been implanted years prior to their death (Fayad et al., 2009; Nadol and Eddington, 2006; Nadol et al., 2001). Therefore it is unlikely that this material will provide insights into postoperative delayed hearing impairment in the foreseeable future. In contrast, experimental animals have a histopathological response to an implant which resembles that seen in humans, with fibrosis, new bone growth (osteoneogenesis) and the presence of foreign body giant cells (FBGCs) being prominent features (James et al., 2008; Xu et al., 1997). Therefore an experimental approach is more likely to provide insights into the aetiology of delayed hearing loss and its relationship to the intracochlear tissue response from implantation.

In our laboratories, we now have a sizeable cohort of guinea pigs that have undergone hearing preservation cochlear implantation, where the nature and extent of the tissue response present within the basal turn of the cochlea has been assessed. These animals received a cochlear implant (CI) into the basal turn with or without a short application of a hearing preservation drug, such as dexamethasone or n-acetyl cysteine. A robust analysis of cochlear histopathology and its impact upon hearing loss has been precluded due to marked variability in the extent of the tissue response in separate experiments. Now, sufficient numbers of animals have been studied across a range of experiments to minimise the influence of inter-animal variability, so an analysis has been conducted incorporating subjects across study groups.

Here we correlate the histopathological appearance of the cochlea, with changes in auditory brainstem response (ABR) thresholds over time in an attempt to determine whether the cochlear histopathology has influenced these hearing outcomes. In all of these experiments there was an initial elevation in ABR threshold, which was still apparent one week after surgery. Over the next three weeks, ABR thresholds either partially recovered, remained unchanged or worsened slightly. These divergent hearing outcomes were correlated with the histopathological analyses performed on cochlear tissue collected four weeks after

implantation. In addition, we examined whether there was a treatment effect upon the tissue response from the drugs applied to these animals.

2. Methods

2.1. Animals

All procedures in this study were approved by the Animal Ethics Committee of the Royal Victorian Eye and Ear Hospital (Projects 05/113A, 06/132A, 07/140A and 07/146A). The guinea pigs were bred in the Biological Resource Centre at the Royal Victorian Eye and Ear Hospital and weighed ≥ 300 g. The anaesthesia used for all ABR testing was 60 mg/kg ketamine and 4 mg/kg xylazine, intramuscularly. For surgical procedures, after induction with ketamine and xylazine, gaseous anaesthesia was titrated against respiration with 0.5–1% isoflurane delivered in oxygen set at a rate of 500 ml/min.

2.2. Experimental groups

The experimental groups have been described in detail previously (Table 1) (Connolly et al., 2010; Eastwood et al., 2010; James et al., 2008; Maini et al., 2009; Souter et al., 2012). In the topical dexamethasone study (James et al., 2008), a carboxymethylcellulose hyaluronic acid polymeric pledget (Seprapak™, Genzyme Corporation) was loaded with 5 μ l of either 2% dexamethasone phosphate or saline and placed on the round window membrane for 30 min prior to cochlear implantation. In the n-acetyl cysteine (NAC) study (Eastwood et al., 2010) Seprapak pledgets were loaded with 5 μ l of either 40 mg/ml n-acetyl cysteine or saline and again applied to the round window membrane for 30 min prior to cochlear implantation. In the systemic dexamethasone study (Connolly et al., 2010), a low dose of dexamethasone (0.2 mg/kg) or saline was injected into the internal jugular vein 60 min prior to cochlear implantation. The experimental groups described above were typified by there having been hearing protection observed at 32 kHz only. In the keyhole limpet hemocyanin (KLH) study (Souter et al., 2012), animals were injected subcutaneously with KLH at a dose of 1 mg a week prior to implantation. KLH-primed animals were then randomised to receive a 5 μ l volume of either 20% dexamethasone or normal saline delivered to the round window on a Seprapak pledget for 30 min prior to cochlear implantation. The animals were given a second dose of KLH a week after implantation. Four weeks after implantation, all animals in

these studies underwent a final ABR measurement and were then euthanised with an overdose of pentobarbitone (1 g/kg) prior to transcardiac perfusion with a phosphate-buffered normal saline solution.

2.3. Auditory Brainstem evoked potential recordings

ABR recordings were completed in the ear to be implanted immediately prior to surgery, then at one and four weeks after surgery (Chang et al., 2009; James et al., 2008). Acoustic stimuli were delivered via a loudspeaker (Richard Allen DT 20, UK) placed 0.1 m from the test ear. The stimuli were computer generated tone pips of 5 ms duration with 1 ms rise/fall times, presented at 2, 8, 16, 24 and 32 kHz. The contralateral ear was masked using an ear mould compound (Otoform K2, DLT, West Yorkshire).

Subcutaneous needle electrodes were placed upon the skull vertex, dorsal neck and midline torso of the guinea pig to record ABRs. Responses were amplified by 100,000× (DAM-5A, W-P Instruments Inc, New Haven, Conn., USA) and band-pass filtered (Krohn-Hite 3750, Avon, Mass., USA) between 150 Hz and 3 kHz (6 dB/octave). The signal was fed to a 16-bit analogue-to-digital converter (Tucker Davis Technologies, USA) and sampled at 20 kHz for 10 ms after stimulus onset then averaged over 250 stimulus repetitions. Stimulus intensity was decreased by 5 dB decrements to sub-threshold levels. The waveforms were analysed using a custom program (software written by Dr James Fallon and adapted by Prof Stephen O'Leary) in Igor Pro 5.02 (Wavemetrics Inc.) with the researcher blinded to the treatment group. Threshold was defined as the lowest stimulus intensity to evoke a wave III response greater than 0.4 μ V.

2.4. Cochlear electrode and implantation

The CI electrode array has been described previously (Chang et al., 2009; James et al., 2008). Its total length was 15 mm, consisting of 3 platinum rings placed 0.75 mm apart along a silastic carrier (MDX4-4210 Dow Corning Products, USA). Platinum wires of 25 μ m diameter were welded to each electrode and embedded within the silastic. The platinum rings served as a depth gauge with insertion to the third ring indicative of 2.25 mm insertion. The maximum diameter was 0.45 mm, narrowing to 0.41 mm towards the tip of the electrode.

Sterility was achieved with ultrasonic cleaning and ethylene oxide sterilisation. The electrode was implanted under general anaesthesia. After making a post-auricular incision, the bulla was exposed and opened with a 3 mm cutting burr. Under magnification, a 0.7 mm cutting burr was used to place a cochleostomy into the basal turn of the cochlea. The silastic-platinum dummy electrode was passed gently into the cochlea using soft surgical principles to the level of the third platinum ring (2.25 mm) and left *in situ*. A fascial graft was used to seal the cochleostomy around the implant. The extracochlear portion was trimmed to fit within the bulla.

2.5. Histological methods

After transcardiac perfusion with phosphate-buffered normal saline, cochleae from all animals were removed and placed in 10% formalin before decalcification in 4% (w/v) ethylenediaminetetraacetic acid (Sigma–Aldrich, USA) for one week. Cochleae were trimmed, the electrode was removed, and the specimen was oriented using agar prior to paraffin embedding and sectioning. When the histologist judged (by visual inspection) that the mid-modiolar region had been reached, three sequential mid-modiolar sections of 5 µm thickness were mounted, cover-slipped, stained with haematoxylin and eosin and examined by light microscopy (Carl Zeiss, Göttingen, Germany).

The position of the tissue response in the lower basal turn of the cochlea was analysed. The ST was divided into four quadrants (see Fig. 1) for the purpose of recording the location(s) of the tissue response and the electrode. The upper inner quadrant (Q1) was proximate to the spiral ganglion and osseous spiral lamina (OSL), the upper outer quadrant (Q2) was in the vicinity of the lateral osseous spiral lamina, basilar membrane and the adjacent lateral wall. Quadrant 3 (Q3) was medio-inferior and quadrant 4 (Q4), latero-inferior. The electrode left a circular lumen in the tissue response that indicated its position prior to electrode removal. Therefore, an electrode position could only be estimated when a tissue response was present. Specific features examined on all sections included the nature of the tissue response, and whether this was constituted of loose areolar fibrosis, dense fibrosis and/or osteoneogenesis. The percentage of ST area occupied by the tissue response was reported. The presence or absence of fracture of the OSL was also noted. The organ of Corti was assessed by counting inner hair cells (IHCs, 0 or 1) outer hair cells (OHCs, 0–3) and the

presence of the tunnel of Corti across the three mid-modiolar sections. The IHC and tunnel were deemed to be present if recognised on one of the three sections examined. OHC counts were averaged across the three sections. The extent of the osteoneogenesis was assessed subjectively, and when present was classed as 0 – absent, 1 – minimal, 2 – occupying up to one quarter of the tissue response, or 3 – occupying greater than one quarter of the tissue response. Spiral ganglion cell (SGC) densities were calculated by dividing the number of Type I SGCs exhibiting an identifiable nucleus by the area of Rosenthal's canal.

2.6. Methods of data synthesis and statistical analysis

In all experiments, timelines for histological analysis and functional testing of the auditory system were standardised. ABR threshold shifts (between preoperative and postoperative levels) were acquired one and four weeks after cochlear implantation. Histological analyses were performed at the four-week postoperative time.

Bivariate analyses (Pearson's correlation) were performed to explore relationships between histological features and/or the ABR threshold shifts. This was followed, where appropriate by multiple regression, to determine whether factors had either collective or unique effects. The treatment groups were ranked according to the value of the threshold shift averaged across frequency (leading to an order from lower to higher of 2% local (KLH), control (KLH), 2% local, NAC, systemic low dose steroid and control) in preparation for multiple regression analysis. A repeated measures analysis of variance was employed to evaluate whether factors such as cochlear turn or treatment group accounted for the variance in ABR thresholds across time or frequency.

Data was collated in Microsoft Office Excel 2010 and statistics were performed in IBM SPSS version 20.

2.7. Rationale for analysis

The primary goal of this study was to investigate whether there were correlations between the histological features within the cochlea after implantation and hearing threshold changes. In order to perform these correlations, the position of histological response

needed to be related to a frequency specific place map. The lower three half turns (lower basal, upper basal and lower second turn) of the cochleae were assessed in mid-modiolar sections (cutting from the round window). Based on the ([Greenwood, 1990](#)) frequency place map, the 32 and 24 kHz frequency responses would have originated from the lower basal, the 16 and 8 kHz responses from the upper basal and the 2 kHz response from the lower second turn. When performing multiple regression, threshold shifts at 32 kHz were used to represent the lower basal turn, 8 kHz to represent the upper basal turn and 2 kHz to represent the lower second turn. Given that the electrode was inserted approximately 2.25 mm into the scala, its tip reached to the cochlear region that gives rise to hearing in the 16–24 kHz range. Therefore, thresholds elevated at 2 and 8 kHz are in a region apical to the tip of the electrode.

3. Results

3.1. Hearing and quantitative analyses of neurosensory elements

At one and four weeks after implantation ABR thresholds were on average 20–30 dB higher for frequencies in the range between 8 and 32 kHz; the threshold shift was greatest at 8 kHz, and was <10 dB at 2 kHz. This can be seen in [Fig. 2](#), which presents the ABR threshold shifts one and four weeks after implantation, with the place-matched histological observations of the organ of Corti (IHC, OHC, presence of the tunnel of Corti) and SGC counts. In the present study, hearing loss that increased in magnitude by 10 dB or more over the four weeks since surgery was seen in 19% of all animals. The IHC, OHC and SGC counts were poorer in the lower basal turn than in either the upper basal or the lower second turns.

Multiple regression was performed to determine factors that may have contributed to the variation in ABR thresholds. For this analysis, IHC, OHC and SGN counts were paired with place-matched ABR thresholds for each of the lower three half turns of the cochlea and entered as dependent variables. Cochlear half turn and treatment were also entered as dependent variables. The multiple regression was weakly predictive of the week four threshold shifts ($r = 0.401$, $r^2 = 0.163$, $p < 0.001$), but only the cochlear (half) turn was found to be a significant factor (beta = -0.337 , $p < 0.001$). This indicated that ABR thresholds

differed across cochlear place, but that neither treatment group, nor place-matched neural and sensory counts explained the variation in ABR thresholds.

3.2. Position and pattern of the tissue response

The cochlear electrode occupied approximately 20% of the area of the ST, and was in most cases surrounded by tissue. The location of the tissue response was determined relative to quadrants of the ST as represented in Fig. 1. The tissue response was most often located laterally, in the vicinity of Q2, or between this quadrant and Q1 (Q1,2) or Q4 (Q2,4). The tissue surrounded a centrally located electrode (Q1,2,3,4) in 11 of the 73 cochleae. Medially placed tissue (Q1 or Q1,3,4) was seen in 4 cochleae only. A tissue response was found within the scala vestibuli in 8 animals. A comprehensive listing of tissue locations is found in the legend of Fig. 1.

The tissue reaction four weeks after implantation was typified by a loose areolar matrix of fibrocytes, which extended from the OSL, the lateral wall, or both, to engulf the electrode (Fig. 3). This tissue typically arose from the cochlear wall nearest to the electrode, and was broadly based along the scalar margin. When the electrode was more centrally located, loose areolar tissue was seen to extend to both the OSL and the lateral wall, often with sparing of the region immediately inferior to the basilar membrane. There was frequently a dense layer of fibrosis, often involving FBGCs, immediately surrounding the electrode. This tissue arose from either the cochlear wall adjacent to the electrode or de novo when the electrode was more centrally placed within the ST. Osteoneogenesis was sometimes present within the tissue response, and varied in extent from being minimal to widespread.

3.3. Relations between histopathological features present within the lower basal turn

The electrode was present in the lower basal turn of the cochlea, and it was here that the majority of the cochlear pathology was found. It was revealed that the percentage of the ST occupied by the tissue response correlated with the presence of FBGCs (Fig. 4a), osteoneogenesis (Fig. 4b), OSL fracture and poorer OHC counts (Table 2). On multiple regression, collectively these factors were predictors of the extent of the tissue response

($r = 0.504$, $r^2 = 0.254$, $p = 0.003$), but only the presence of FBGCs was demonstrated to have a unique effect ($\beta = 0.263$, $p = 0.045$).

OHC survival in the lower basal turn could be excellent, even in the presence of a tissue response within the ST. In fact, a full complement of OHC was present in 53% of cochleae analysed. However, OHC counts were negatively correlated with the extent of tissue response, the extent of new bone growth and the presence of FBGCs ([Table 2](#)), and while on multiple regression the collective effect of these factors (on OHC count) was not significant ($p = 0.071$) the unique effect of osteoneogenesis was ($p = 0.026$).

3.4. Effect of treatment group upon the tissue response

A multivariate ANOVA was performed to determine whether the treatment group had an effect on the variability of the tissue-related factors. Treatment group was found not to have an effect upon either the extent of the tissue response ($p = 0.310$), the presence of foreign body giant cells ($p = 0.071$) or OSF fracture ($p = 0.288$). Similarly, there was not a significant effect of treatment group upon SGC counts in either the lower basal ($p = 0.547$), upper basal ($p = 0.115$), lower second ($p = 0.072$) or upper second ($p = 0.461$) turns. However, treatment group did have a significant effect upon OHC counts in the lower basal turn ($p < 0.001$), the upper basal ($p = 0.028$) turn and the upper middle ($p = 0.045$) turn. Post hoc testing revealed that this effect could be accounted for by NAC-treated cochleae having significantly lower OHC counts than other treatment groups in the basal turn.

3.5. Relationships between hearing, neurosensory counts and the tissue response in the lower basal turn

It was revealed in Section [3.1](#) that the variability in ABR thresholds was not predicted by place-matched neurosensory counts. This means that the survival of hair cells and SGCs did not account for the elevated ABR thresholds in the 8 kHz region, apical to the location of the electrode. As described above, theories of hearing loss after cochlear implantation predict either implant trauma and/or the tissue response to implantation, so here we explore whether there are relationships between the cochlear histopathology in the lower basal

turn of the cochlea and ABR thresholds and/or the integrity of neurosensory structures within the lower two cochlear turns.

There was a relationship between the percentage of the ST occupied by the tissue response and the week four threshold shifts at 8 and 16 kHz (Table 3). The 16 kHz data was plotted in Fig. 5 where it is apparent that large threshold shifts were sometimes observed even when the tissue response was minimal, but the converse was not observed. This pattern of response was apparent from 2 to 32 kHz (data not shown). The week four threshold shifts correlated with osteoneogenesis at 8 and 24 kHz (Table 3). OSL fracture correlated with threshold shifts at 8, 16 and 32 kHz. The change in threshold between one and four weeks after surgery correlated with the percentage of the ST occupied by the tissue response and osteoneogenesis at 8 and 16 kHz (Table 3). Fig. 6 shows the data at 16 kHz; hearing recovered (negative values) only when the tissue response occupied less than 50% of the ST. If the tissue response was more extensive, hearing either did not recover or deteriorated further (positive values).

Fewer correlations were found between the tissue response and the neurosensory counts in the lower two (full) cochlear turns. Those observed were between osteoneogenesis and IHC, OHC and SGC counts in the lower basal turn ($r = -0.441, -0.368$ and -0.403 , respectively, $p < 0.01$); OSL fracture and IHC, OHC and SGC counts in the lower basal turn ($r = -0.410, -0.356, -0.325$, respectively, $p < 0.01$) and OSL fracture and OHC counts ($r = -0.349, p < 0.01$) in the upper basal turn. These findings demonstrated that osteoneogenesis or OSL fracture in the basal turn negatively impacted upon more apical neurosensory counts, particularly in the upper basal turn. The percentage of the ST occupied by the tissue response did not correlate with any of the more apical neurosensory counts.

4. Discussion

Here we have shown that the tissue response to the CI was confined to the lower basal turn, where the electrode resided. Aspects of this tissue response, such as its extent, the presence of OSL fracture, FBGCs, and osteoneogenesis, correlated with poor ABR thresholds both in the basal turn and also at more apical regions. Metrics relating to neurosensory structures, namely the IHC, OHC and SGC counts, correlated with the tissue response in the

lower basal turn, but fewer correlations were found with the neurosensory structures in the more apical turns. These observations help to explain why place-matched IHC, OHC and SGC counts were not significant covariates for ABR threshold shifts on repeated measures ANOVA; the organ of Corti was substantially preserved on light microscopic examination in the upper basal turn and above, while ABR thresholds were significantly elevated.

The treatment groups did not have a main effect on either the ABR thresholds or the tissue response, presumably because the studies selected for this analysis involved treatments leading to a minimal effect on hearing, and when present was confined to 32 kHz. We attribute the lack of a treatment effect in the local steroid studies to the relatively short, 30 min period of drug application to the round window (Chang et al., 2009). We have subsequently shown that application periods of 60–120 min are required to cause effective hearing protection (Chang et al., 2009). The low dose systemic steroid group included in this analysis was not effective in preserving hearing. Effective hearing protection required a higher dose of systemic steroid. The pooling of control animals across studies may have masked some of the treatment effects seen at 32 kHz noted in previous studies.

An important observation of this study is that four weeks after surgery lower ABR threshold shifts were not observed when there was extensive fibrosis. This is apparent from Fig. 5 where all the data points were in the lower right triangle of the graph, demonstrating that significant hearing loss can be associated with minimal fibrosis, but not the converse. Furthermore, there was an almost (log-)linear relationship between the best hearing thresholds at any level of fibrosis, above ~40% fibrosis. The latter suggests that fibrosis and hearing may be related, either directly or indirectly. One possibility is that fibrosis reflects the extent of intracochlear inflammation in the postoperative period that in itself causes greater hearing loss (an inflammatory theory) (Eshraghi et al., 2005). Another possibility is that fibrosis reduces hearing thresholds by a direct effect upon cochlear mechanics, as suggested by Choi (a mechanical theory) (Choi and Oghalai, 2005). These potential mechanisms and their relative merits are now considered.

In the experimental setting, the cochlear inflammation provoked by CI surgery has been shown to cause hearing loss by promoting oxidative stress and apoptosis within hair cells (Dinh et al., 2011; Eshraghi et al., 2006; Haake et al., 2009). This occurs not only at the site

of injury but also at remote cochlear locations, which provides a potential explanation for the low frequency hearing loss observed following cochlear implantation. It is plausible that cochlear inflammation may also determine the extent of the tissue response by influencing cellular recruitment into the cochlea after injury; greater inflammation will attract larger numbers of immune competent cells into the cochlea (Tornabene et al., 2006), including the macrophages and fibroblasts required for the generation of the tissue response. Therefore, it may be proposed that the severity of the inflammatory response determines both the magnitude of the hearing loss and also the extent of the tissue response, both being different manifestations of the same underlying mechanism. Several observations can be made that may be consistent with this interpretation. First, consider the association between the extent of the tissue response and the presence of FBGCs (Fig. 4a). FBGCs arise when macrophages are unable to phagocytose foreign material, as is the case following cochlear implantation (Anderson et al., 2008). The inflammatory theory predicts that the severity of the inflammatory response will determine the numbers of macrophages recruited into the cochlea and thus the chance that these will coalesce into FBGCs, and this is consistent with our observation that a greater percentage of ST was occupied by fibrosis when FBGCs were seen. The presence of FBGCs within the tissue response did not affect the ABR thresholds significantly although there was a trend in that direction, suggesting that these may not be related directly, but more likely indirectly through inflammation. Interestingly, a reanalysis of the data presented by Xu and Shepherd (Xu et al., 1997) when cochlear electrodes were implanted into the hearing cat supports this association. In that study, ABR thresholds were recorded in response to an acoustic click and these were poorer when FBGCs were seen in the tissue response (Kruskal–Wallis, $p < 0.01$).

In the present study, a relationship between fibrosis and inflammation is supported by the significant negative correlation between OHC counts and fibrosis. A more extensive tissue response also correlated with more extensive new bone growth, and both were correlated with OSL fracture. It is tempting to suggest in the present study that when OSL fracture occurred this led to a more extensive inflammatory response provoking both an extensive tissue response and also OHC loss. However, in the present study we cannot exclude the possibility that OSL fracture may lead to OHC loss by other mechanisms, such as vascular compromise through disruption of the spiral cochlear vein.

While the ABR responses appear to be consistent with the idea that inflammatory changes initiated at the site of electrode insertion extended to more apical cochlear regions, the paucity of corresponding changes in the histopathology in the upper basal and second turns might suggest a more complex interpretation of these data. There was no evidence of fibrosis in the cochlear half turns apical to the electrode, and IHC and OHC counts were close to normal. Therefore, the elevation in ABR thresholds observed at 8 kHz, that arose from the upper basal turn, must have arisen from a structural change in the hair cells, disruption of the afferent synapse or a change in cochlear mechanics.

Models of passive cochlear mechanics (Choi and Oghalai, 2005; Kiefer et al., 2006) have demonstrated that if fibrosis were to increase damping of the cochlear partition by even a modest degree of 5–6 times then there would be a substantial reduction in basilar membrane velocity in the region involved, resulting in an elevation in high frequency hearing thresholds for CI recipients. There is clinical evidence to support this in patients, since pure tone thresholds may improve at frequencies just apical to the position of the cochlear electrode (Kiefer et al., 2006). Alternatively, if the tissue response surrounding the electrode is more extensive and increases the magnitude of damping within the ST by 2–3 orders of magnitude, then there is predicted to be an elevation in lower frequency hearing (Choi and Oghalai, 2005). Fig. 5 revealed that above ~40% fibrosis within ST, there was an almost (log-)linear relationship between the extent of tissue response and the best hearing observed across all animals and frequencies. This is in keeping with Choi's modelling both qualitatively and quantitatively, and consistent with an interpretation that the fibrosis causes significant damping. The correlation between fibrosis and the recovery of ABR thresholds over weeks one to four demonstrates that good recovery of hearing is possible only when there is a less extensive tissue response; when there is greater tissue response, hearing recovers only a little and sometimes worsens over time. This observation could potentially be explained by the effects of progressive cochlear damping as the tissue response matured.

It is known that electrode trajectory and electrode design influence direct trauma to the basilar membrane or OSL (Briggs et al., 2001). The present study demonstrates how detrimental OSL fracture is in affecting hearing loss and leading to an extensive tissue

response. These results strongly support the proposition that OSL injury must be avoided when attempting hearing preservation surgery. Hearing loss was clearly more likely with perimodiolar electrode placement, and leads the authors to suggest that with currently available electrodes that lateral placement may be preferable for hearing preservation.

In the present study, hearing loss that increased in magnitude by 10 dB or more over 4 weeks was seen in 19% of all animals. This is a similar percentage to that seen with delayed hearing loss in the clinical situation, where the incidence ranges from 20 to 30% between studies. While it must be remembered that delayed hearing loss in humans can arise months after implantation, our observation that progressive hearing loss in this animal model was associated with a more extensive tissue response may be of interest when attempting to understand the clinical situation.

5. Conclusions

Improving the preservation of residual hearing after CI surgery, and in particular preventing the delayed hearing loss seen in over 25% of patients, requires a more thorough understanding of the relationship between the cochlear response to implantation and hearing. Such an understanding will facilitate the rational development of hearing protection therapies, and help to further refine electrode design specifications. Here we present evidence that the best possible postoperative hearing is limited by the extent of the tissue response. This is consistent with the mechanical theory of hearing impairment and suggests that reducing the extent of the tissue response is a worthy strategy for future research aimed at protecting hearing after implantation. Alternatively, the data presented is also consistent with an interpretation that the tissue response is related to inflammation and cochlear injury, as suggested by its relationship with the presence of FBGCs, osteoneogenesis, OHC loss and OSL fracture. Whether one or both mechanisms contribute to hearing loss will require further experimentation. However, from the available evidence it would be prudent to recommend that soft-surgery and inflammation reduction strategies should be considered for minimising the extent of the tissue response. Progressive hearing loss also correlates with the extent of the tissue response, so reducing the tissue response may be an effective strategy for preventing delayed hearing loss in patients after implantation.

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Table 1

Summary of treatments applied to the animals included in this analysis. All animals underwent cochlear application. Some were pre-treated with either topical application of drug to the cochlea, via its application to the round window on a carboxymethylcellulose-hyaluronic acid polymer. Others were treated systemically, via intravenous injection. "KLH" animals were inoculated to induce an immune response against keyhole limpet hemocyanin.

Study	Treatment (all received implant)	Numbers	Descriptor
1. Topical dexamethasone (James et al., 2008)	Local application to round window 2% dexamethasone	14	2% dex
2. Topical NAC (Eastwood et al., 2010)	Local application to round window 200 µg n-acetyl cysteine	14	NAC
3. Systemic dexamethasone (Connolly et al., 2010)	Intravenous administration 0.2 mg/kg dexamethasone	6	Low dose
4. KLH/Topical dexamethasone (Souter et al., 2012)	Local application to round window 20% dexamethasone KLH primed	10	KLH steroid
5. KLH control (Souter et al., 2012)	Local application to round window normal saline KLH primed	10	KLH control
6. Controls from non-KLH studies		19	Control
Total		73	

Table 2

Correlation co-efficients between characteristics of the histopathology in the lower basal cochlear turn. The histopathological measures were the percentage of scala tympani occupied by the tissue response (% Tissue in ST), the presence of foreign body giant cells (FBGCs present), the extent of the osteoneogenesis (New Bone), fracture of the osseous spiral lamina (OSL Fracture), inner hair cell counts in the lower basal turn (IHC Counts) and outer hair cell counts in the lower basal turn (OHC Counts). Significant correlations are bolded; *significant to 0.05, **significant to 0.01.

	% Tissue in ST	FBGCs present	New bone growth	OSL fracture	IHC counts	OHC counts
% Tissue in ST	1	0.414**	0.478**	0.365**	-0.214	-0.175
FBGCs present		1	0.411**	0.255*	-0.16	-0.123
New bone			1	0.717**	-0.441**	-0.368**
OSL fracture				1	-0.410**	-0.356**
IHC counts					1	0.916**
OHC counts						1

Table 3

Correlation co-efficients between the histopathology of the lower basal turn and ABR threshold shifts at four weeks. The histopathological measures were the percentage of scala tympani occupied by tissue (% Tissue in ST), the extent of the osteoneogenesis (New Bone Growth), the presence of foreign body giant cells (FBGCs present), outer hair cell counts in the lower basal turn (OHC Counts) and fracture of the osseous spiral lamina (OSL Fracture). Significant correlations are bolded; *significant to $p < 0.05$, **significant to $p < 0.01$.

	Threshold shifts 4 weeks after surgery				
	32 kHz	24 kHz	16 kHz	8 kHz	2 kHz
% Tissue in ST	-0.04	0.095	0.279*	0.339**	0.141
New bone growth	0.237	0.276*	0.175	0.351**	0.064
FBGCs present	-0.181	-0.063	0.113	0.331**	-0.102
OHC counts	-0.206	-0.275*	-0.237	-0.386**	-0.035
OSL fracture	0.250*	0.245	0.339**	0.266*	0.061

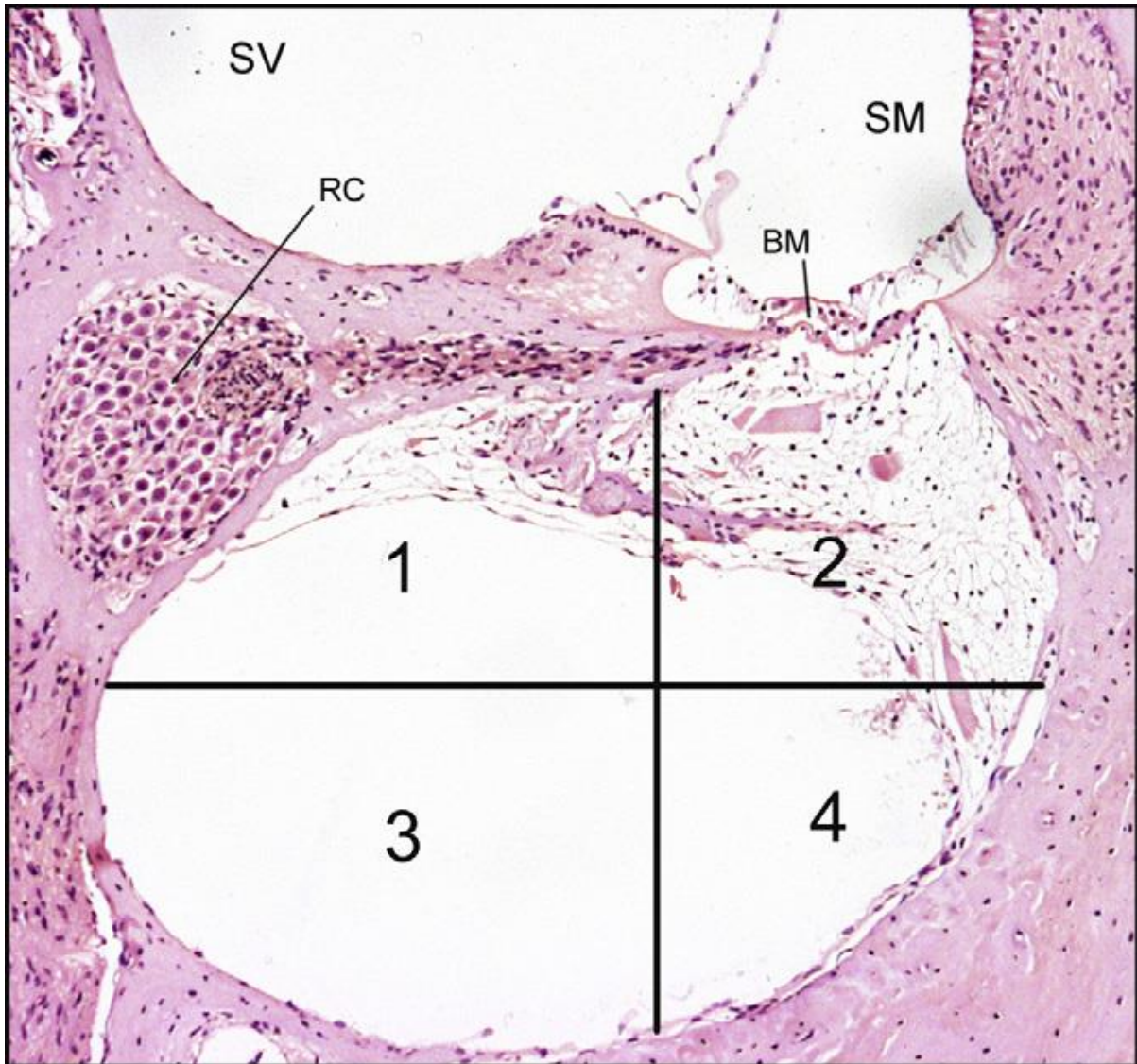


Fig. 1. The scala tympani (ST) was divided into quadrants (1, 2, 3, and 4) as shown in order to qualitatively describe the electrode position and tissue response. The scala vestibule (SV), scala media (SM), basilar membrane (BM) and Rosenthal's canal (RC) are marked. The tissue response was found in quadrants 2 and 4 (Q2,4) in 20 animals, Q1,2,4 in 12 animals, Q1,2,3,4 in 11, Q1 in 4, Q2 in 3, Q1,2 in 3, Q4 in 1, Q1,3,4 in 1 and no tissue response was evident in 10. The tissue response was found in SV in 3, and both SV and ST in 5.

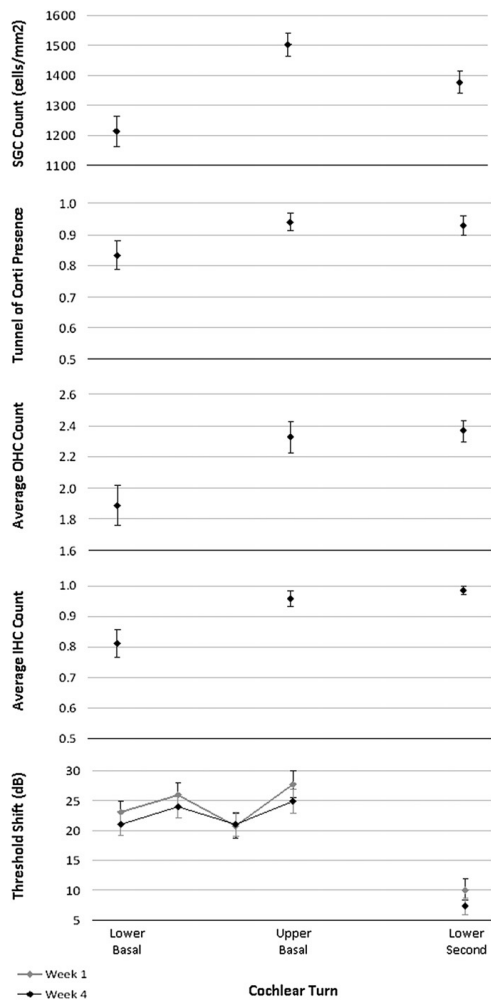


Fig. 2. Summary of the histopathological findings and auditory brainstem threshold shifts from the lower basal to the lower second cochlear turns. The lower panel presents threshold shifts from the lower basal turn (from left to right, 32, 24 and 16 kHz), the upper basal turn (8 kHz) and the lower second turn (2 kHz). The thresholds at one and four weeks after surgery are presented. The inner hair cell (IHC), outer hair cell (OHC) and spiral ganglion cell (SGC) counts from each cochlear half-turn are also presented. The presence of the Tunnel of Corti was recorded as either present (1) or absent (0), and the values were averaged to generate the values for the Figure Bars represent the standard error of the mean.

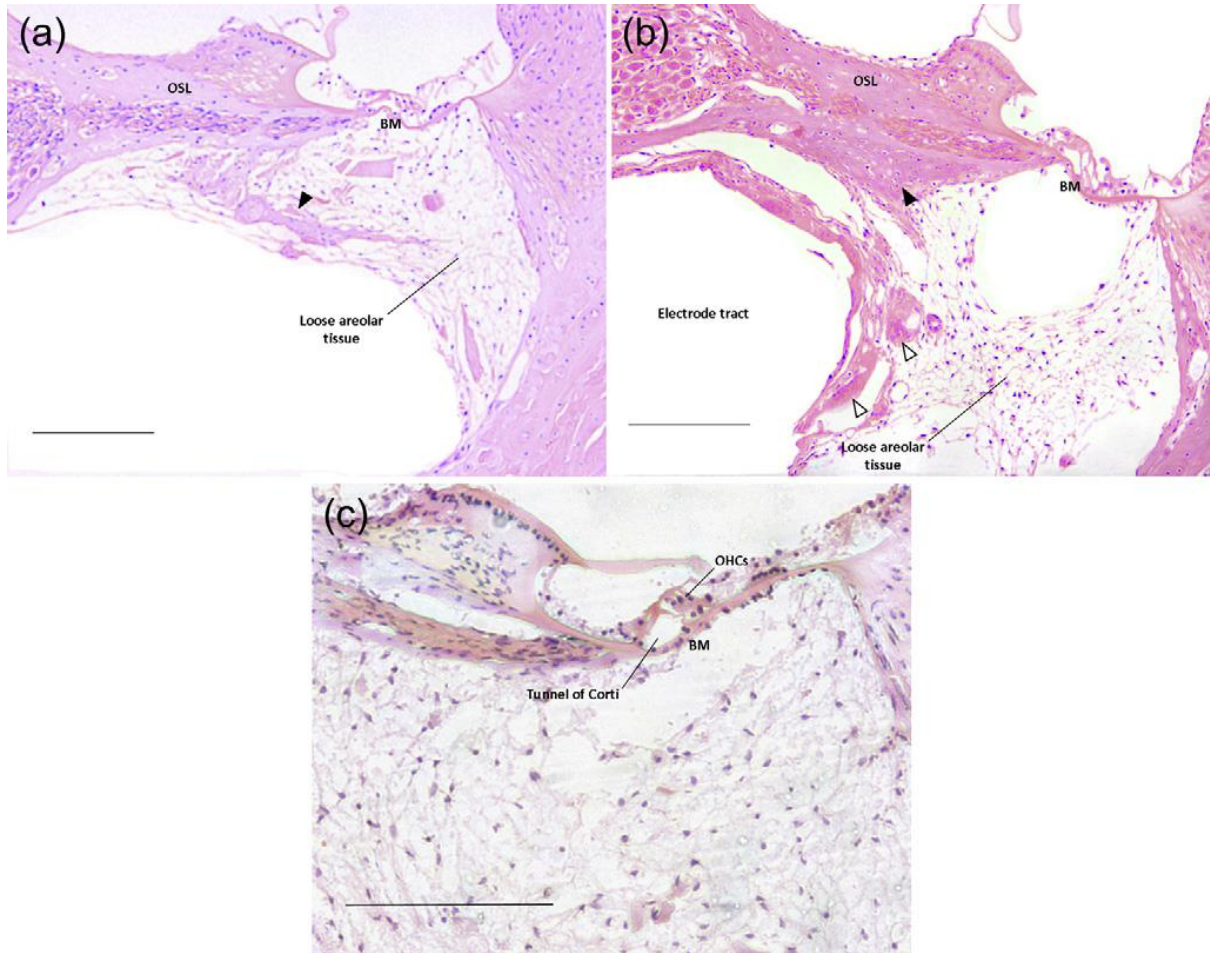


Fig. 3. Examples of cochlear histopathology. Bar: 200 μ m. (a) Loose areolar tissue extends from the osseous spiral lamina (OSL) and the lateral wall, with some new bone growth (black arrow head). The outer hair cells are present. (b) There is a dense fibrotic reaction surrounding the electrode, associated with some foreign body giant cells (white arrow heads). Significant new bone growth extends from the osseous spiral lamina (black arrow head). There is loose areolar fibrosis extending from the OSL and lateral wall, with sparing of the region below the basilar membrane (BM). (c) Extensive loose areolar tissue extending from the scalar walls. Note the presence of a full complement of outer hair cells, with preservation of the tunnel of Corti.

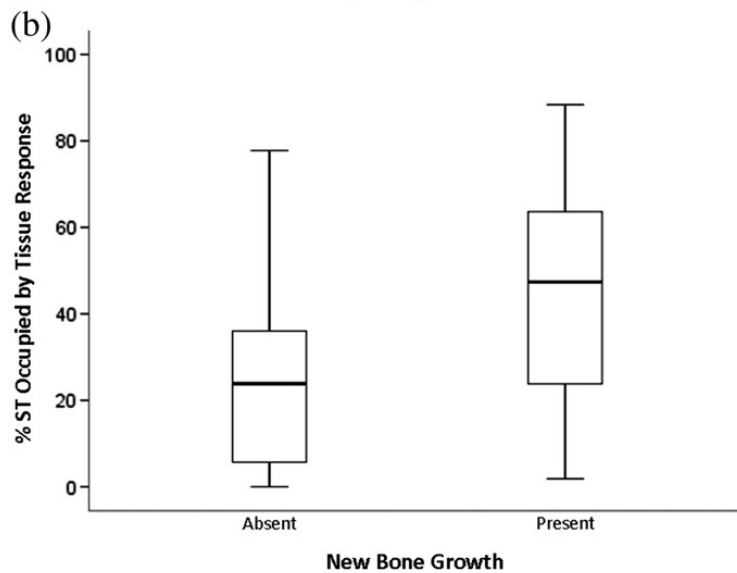
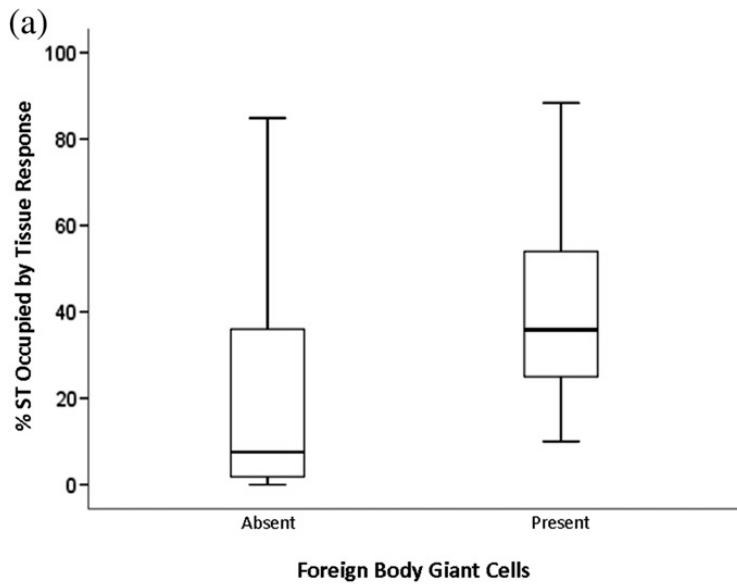


Fig. 4. (a) the percentage of scala tympani (ST) occupied by the tissue response is plotted depending upon whether foreign body giant cells were observed, or not. The box plot defined quartiles, 90% range and outliers. (b) The same data as (a) but with the scala percentage grouped by the presence or absence of new bone growth being detected in the tissue response. Here the “presence” of osteoneogenesis equates with a grading of 1e3 as outlined in Section 2.5, i.e. minimal to extensive.

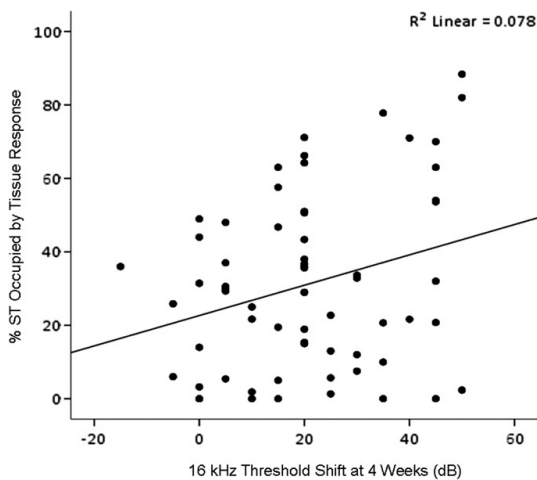


Fig. 5. The percentage of scala tympani (ST) occupied by the tissue response is plotted against the auditory brainstem response threshold shift (dB) at 16 kHz four weeks after implantation.

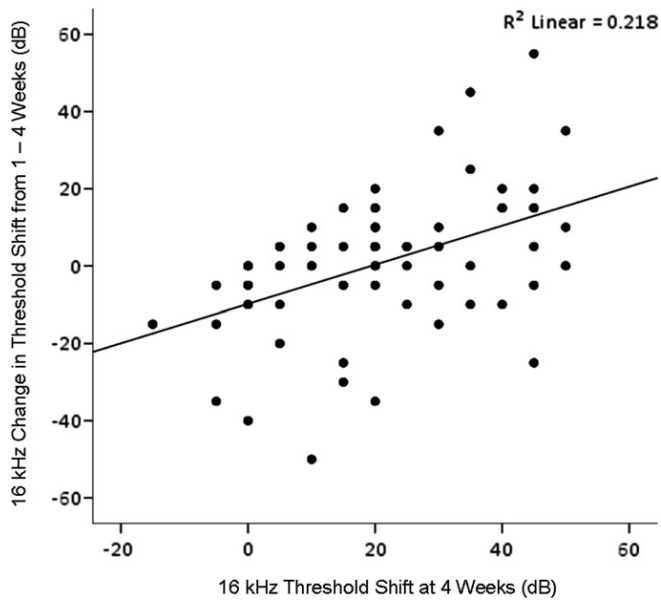


Fig. 6. The auditory brainstem response (ABR) threshold shift (dB) at 16 kHz four weeks after implantation is plotted against the change in ABR thresholds between weeks one and four after implantation (dB) e positive values indicate deterioration in thresholds.