Seventh Quarterly Progress Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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1. Introduction

The goal of this contract is to develop methods of protecting the remaining portions of the auditory system from degeneration after loss of hair cells and to improve its effectiveness in extracting information provided by auditory prostheses. We have taken a broad neurobiological approach to this goal in order to study both the short and long-term response of the auditory system to loss of hair cells and the subsequent introduction of afferent input via an auditory prosthesis. Our studies are divided into three major areas of investigation:

(a) The neurophysiological and neuroanatomical response to prolonged electrical stimulation of the auditory nerve following a neonatal sensorineural hearing loss (SNHL). This work is designed to provide insight into the protective effects of electrical stimulation on the auditory nerve (AN) in addition to investigating the plastic response of the central auditory system (CAS) to temporally challenging stimuli presented chronically to one or two sectors of the AN.

(b) The neurophysiological and neuroanatomical response to the AN and CAS of deafened animals following prolonged intracochlear electrical stimulation in combination with neurotrophic support of the auditory nerve. This work is designed to investigate whether electrical stimulation and chronic administration of neurotrophins act in synergy to promote AN survival. This work will also provide insight into the role of neurotrophins in improving synaptic efficiency in the deafened auditory pathway.

(c) The neurophysiological and neuroanatomical response to acute electrical stimulation of the auditory nerve following a neonatal SNHL. These studies are designed to provide insight into the acute response of the AN and CAS to intracochlear electrical stimulation in deafened animals with little prior auditory experience.

While these studies are designed to provide insight into the plastic response of the deaf auditory pathway to re-activation via an auditory prosthesis, a major objective of this work is to apply our findings to the clinical environment.

2. Summary of activities for the quarter

During the seventh quarter of this contract the following activities were completed:

- Completed our terminal acute electrophysiological studies on the first group of 15 chronically stimulated cats.
- Completed the software and protocol development for the histological examination of cat and guinea pig cochleae and commenced histological analysis. The software allows graphic reconstruction of the cochlea for construction of cochleograms (Schuknecht, 1953).
- Continued to manufacture guinea pig electrode arrays with chronic delivery systems (see *First Quarterly Progress Report).* A problem with a batch of

PVC tubing was detected and resolved. Details of this work will be presented in a future report.

- Deafened six adult guinea pigs and completed the chronic stimulation program and terminal acute electrophysiological study of eight animals. Details of this work will be presented in a future report.
- Continued deafening and preparing rat cochleae and CNS tissue for ongoing neuroanatomical and neurochemical studies of the deafened auditory system (see *Fourth Quarterly Progress Report).*

3. Chronic electrical stimulation studies in neonatally deafened cats

A major undertaking during the quarter was the continuation of the terminal electrophysiological experiments we commenced mid 2001 (*Fourth Quarterly Progress Report*). We performed an additional six experiments during the quarter, bringing the total number of experiments completed to fifteen. Fourteen of the animals had been chronically stimulated for periods of up to eight months. The fifteenth animal was the first of our cohort of deafened, un-stimulated controls (Table 1). Terminal experiments for the remaining seven control animals will be completed during the last quarter of 2002 and the first quarter of 2003.

This report will provide a detailed analysis of the first four chronically stimulated cats. We present details of the chronic stimulation program, including longitudinal electrode impedance and acoustic (ABR) and electrically evoked auditory brainstem responses (EABRs). In addition, we present a description of our histological techniques and analysis of the cochleae from our first four animals. A detailed account of the acute electrophysiological experiments will be presented in a future report.

3.1 Chronic stimulation program

Portable programmable stimulators developed “in house” were used in the present study. The stimulus regime is illustrated in Fig. 1. Briefly, the stimuli consist of 100 μs/phase charge balanced biphasic pulses and are delivered to a bipolar electrode configuration. Direct current (DC) is minimized using capacitive coupling and electrode shorting techniques (Huang et al., 1999). Stimuli are presented at 12000 pulses per second (pps) and are amplitude modulated at a depth of 50% at 30 Hz. The minimum stimulus intensity is set at EABR threshold for that electrode pair; the maximum stimulus intensity is 6 dB above the EABR threshold.

These portable constant current stimulators are carried in a harness worn by the cat. The animals receive approximately 7 h of stimulation per day. Both current intensity and electrode voltage is measured twice daily (Fig. 1); electrode impedance is calculated from these data and monitored longitudinally (Fig. 2). A description of the electrode arrays used is given in our *Third Quarterly Progress Report*; electrode 1 is the most apical and electrode 8 the most basal electrode on the array. The duration of deafness, total implantation period, and the total stimulation time of the four animals described in this report are presented in Table 2.

Table 1. Summary of pre-operative hearing thresholds (in dB SPL). Click thresholds were determined for both ears while frequency specific ABRs were determined unilaterally.

Animal	1 kHz	2 kHz	4 kHz	8 kHz	Click (L R)	terminal experiment
NDC_1	**-**	**-**	**-**	**-**	**>98 >98**	√
NDC_2	-	-	-	-	>98 >98	√
NDC_3	**63**	**71**	**91**	**>93**	**83 63**	√
NDC_4	**38**	**41**	**66**	**93**	**58 48**	**no experiment**
NDC_5	-	-	-	-	>98 >98	√
NDC_6	58	81.5	86	>93.5	72 -	√
NDC_7	**43**	**56.5**	**76**	**77.5**	**57 -**	√
NDC_8	-	-	-	-	>98 >98	√
NDC_9_c		66.5	>91	>93	83 83	√
NDC_10	N/A	N/A	N/A	N/A	88 88	√
NDC_11	N/A	N/A	N/A	N/A	73 78	√
NDC_12	N/A	N/A	N/A	N/A	88 88	√
NDC_13	N/A	N/A	N/A	N/A	93 >98	√
NDC_14	-	-	-	-	>98 >98	√
NDC_15	-	-	-	-	>98 >98	√
NDC_16	-	-	-	-	>98 >98	√
NDC_17_c	N/A	N/A	N/A	N/A	63 68	on going
NDC_18_c	-	-	-	-	>98 >98	on going
NDC_19_c	-	-	-	-	>98 >98	on going
NDC_20_c	N/A	N/A	N/A	N/A	53 53	on going
NDC_21_c	N/A	N/A	N/A	N/A	68 73	on going
NDC_22_c	-	-	-	-	>98 >98	on going
NDC_23_c	-	-	-	-	>98 >98	on going

Notes: c: non-stimulated control animal; N/A: data not available; - no response at maximum intensity tested. In some graphs animals may be identified using a different nomenclature; NDC_1 as 901; NDC_2 as 902 etc. The four animals described in the present report are highlighted.

Current and electrode voltage waveforms

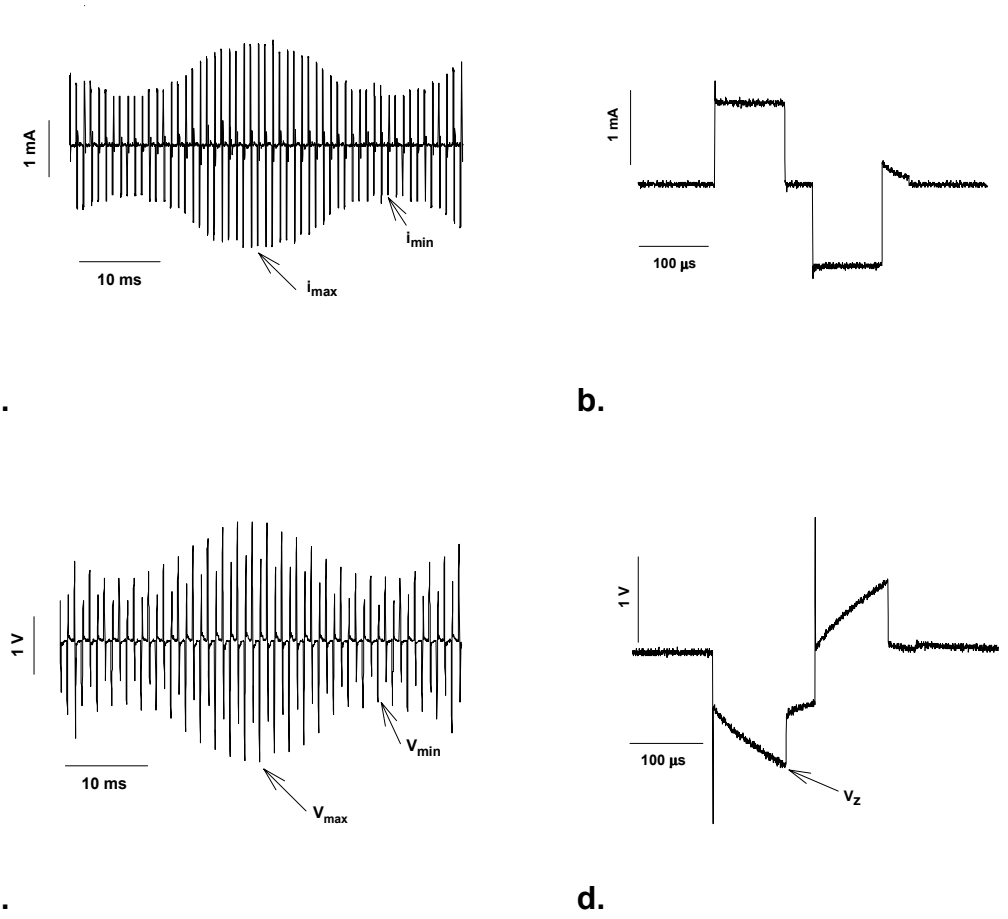


Figure 1. Example of the stimulus waveform used in the present study. Top panels illustrate the current waveform at a wide (a) and narrow (b) time base while the bottom panels illustrate the corresponding electrode voltage waveforms. The stimulus consists of a 1200 pulses per second carrier, amplitude modulated to a depth of 50% at 30 Hz. The current pulses (b) are 100 µs charge balanced biphasic pulses with a 14 µs interphase gap. Additional charge balancing is achieved using capacitive coupling and electrode shorting to ensure minimal DC levels within the cochlea (Huang et al., 1999). Note that the figure illustrates one of two programmable channels that the portable stimulator can output. The variable height of the voltage waveform in (c) is a result of aliasing.

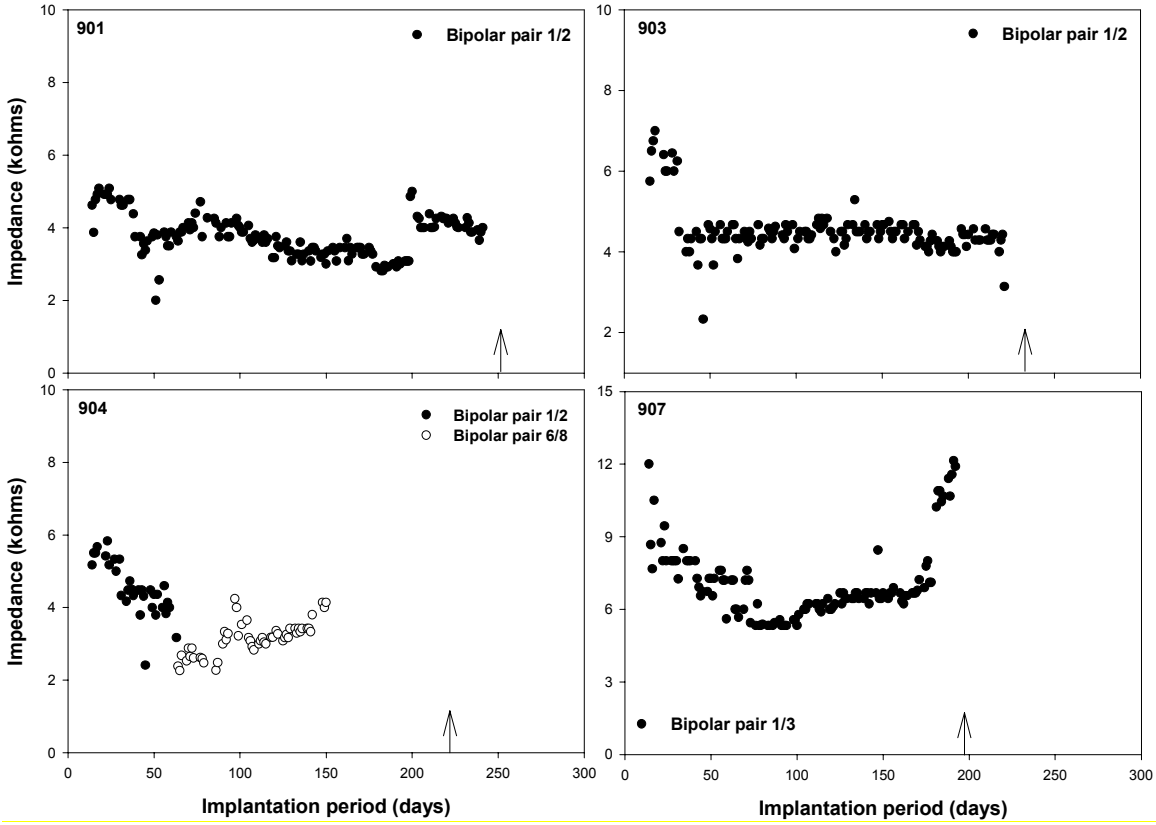


Figure 2. Electrode impedance measured as a function of implantation period in days. Impedance measurements were performed on a daily basis for the duration of the stimulation program. These data were first collected approximately 10 days following surgery when the stimulation program commenced. The arrow indicates when the animal was sacrificed. In some instances (e.g. NDC_4), there was a relatively long period between the end of stimulation and sacrifice. This was included in our protocol in order to examine for long-term effects of neural protection and plasticity after the stimulus had been removed. Note the variation in the scale of the ordinate. The data illustrated here are derived from $Z_{max}=V_{max}/I_{max}$ (see Fig. 1).

Table 2. Summary of duration of deafness and implantation period for the four animals completed to date.

Cat	Duration of deafness (days)	Implantation period (days)	Total stimulation time (hours)
NDC_1	307	252	1093
NDC_3	290	238	930
NDC_4	276	223	567
NDC_7	236	195	832

3.2 Longitudinal hearing status of severely deafened animals

Eight of our cohort of 16 chronically stimulated animals have partial hearing (Table 1) and are classified as severe rather than profoundly deaf. These animals were included in our study to model the severely deaf cochlear implant patient, which now forms a significant proportion of implant subjects.

In addition to regular EABR monitoring of all our stimulated animals (see below), we also monitor the hearing status of the severe hearing loss group. Three of our first four animals exhibited small levels of hearing (e.g. Fig. 3). Their longitudinal click-evoked ABR thresholds are illustrated in Fig. 4. Recording details can be found in Hardie and Shepherd (1999).

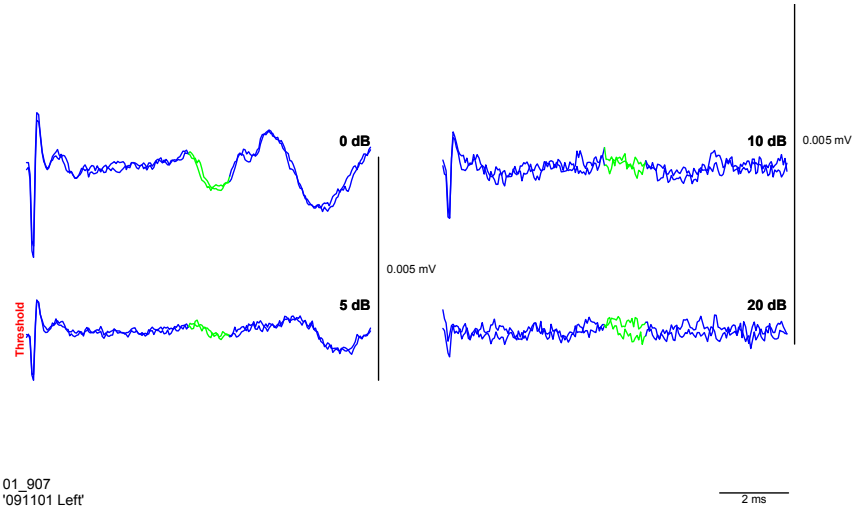


Figure 3. Examples of click-evoked ABRs evoked from the left ear of NDC_7 approximately 5 months following cochlear implantation. The hearing status of the severely deafened animals in this study was monitored longitudinally throughout the stimulation program. The stimulus intensity indicated is dB attenuation re a 98 dB acoustic click.

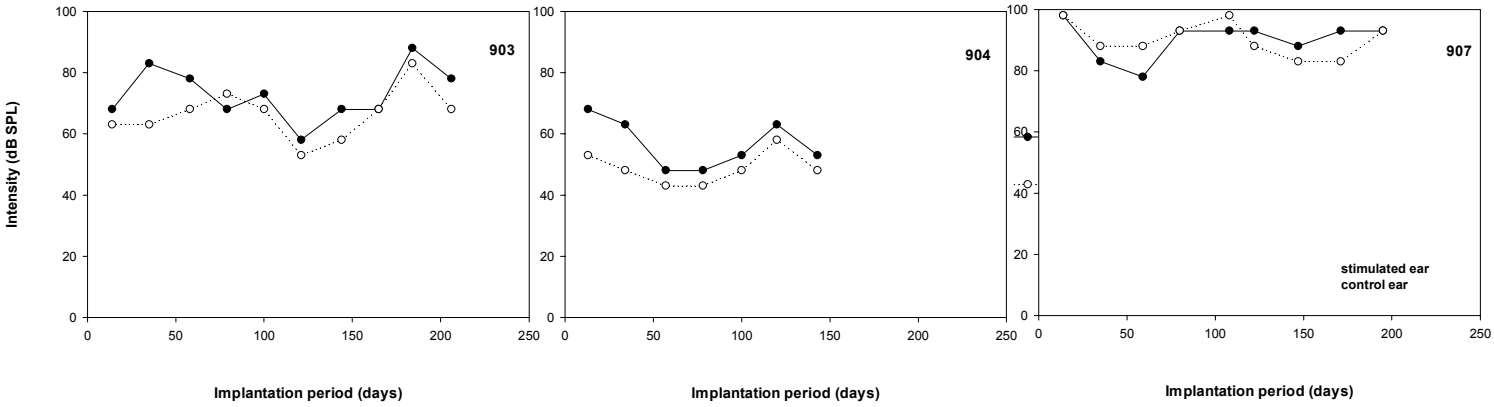


Figure 4. Click-evoked ABR thresholds as a function of implantation time. Open symbols illustrate data from implanted un-stimulated control cochleae while closed symbols are from stimulated cochleae.

3.3 Electrically-evoked auditory brainstem responses

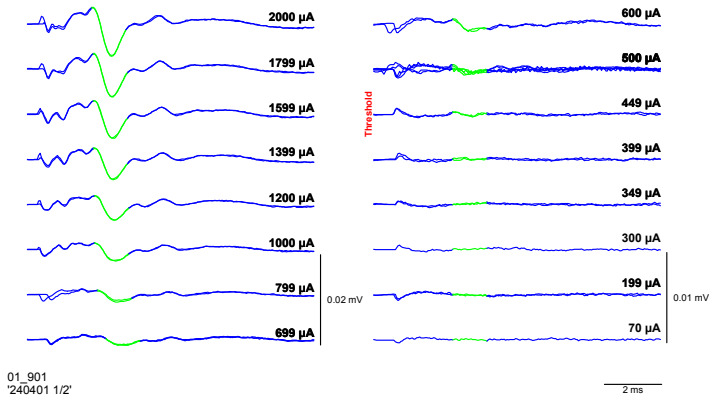
EABRs were recorded approximately every three weeks during each animals chronic stimulation program. These responses were used as an electrophysiological monitor of the status of the auditory nerve and brainstem; they provided an objective measure of whether or not the chronic stimulus was operating at supra-threshold current levels; and threshold estimates allowed us to regularly adjust each animal's stimulator to ensure the minimum current level corresponded to EABR threshold. Representative EABRs recorded (i) just prior to stimulation and (ii) on completion of the stimulus program are illustrated in Figs. 5-8. EABR input/output (i/o) functions, illustrating the response amplitude of wave IV versus stimulus intensity, are illustrated in Fig. 9.

3.4 Cochlear histology techniques

On completion of the acute electrophysiological experiment each animal was killed with an overdose of anesthetic, systemically perfused with fixative, and the cochleae harvested for histology using the techniques of Xu et al., (1997). Following trimming and decalcification, the cochleae were embedded in resin and sectioned at a thickness of 2 μm in the horizontal plane. Sections every 125 μm were stained with hematoxylin and eosin.

The histopathological assessment of each cochlea involved examining all sections serially. The inter-basilar membrane distance was measured (Fig. 10) and the cochlea graphically reconstructed (see below). Cochleae were serially examined for evidence of fibrous tissue and new bone formation within the scala tympani (% estimate of the scala tympani area); presence or absence of inner and outer hair cells and the structure of the organ of Corti; and for evidence of electrode insertion trauma. A cochleogram of the resultant cochlear histopathology was plotted as a function of the distance along the basilar membrane.

In order to quantify the extent of the spiral ganglion cell loss, and to statistically evaluate the potential protective effects of electrical stimulation, cell densities were calculated in each cochlear turn containing a clear Rosenthal's canal. All ganglion cells containing a nucleus were counted; the total count was divided by the area of Rosenthal's canal to obtain a measure of cell density. In order to minimize bias, counting was performed by a single, experienced observer; the counting was undertaken "blind" – the observer was unaware of the identification of each section; and the sections were examined in a random order. Mean spiral ganglion cell densities were then calculated for (i) the lower basal turn (0-10% of the basilar membrane); (ii) the upper basal turn (10-50%); (iii) middle turn (50-75%) and (iv) the apical turn (75-100%). The electrode array was typically inserted to 40-45% of the basilar membrane length. The spiral ganglion cells within the upper basal turn were, therefore, adjacent to the stimulating electrodes and would be subject to the most extensive electrically evoked neural activity.



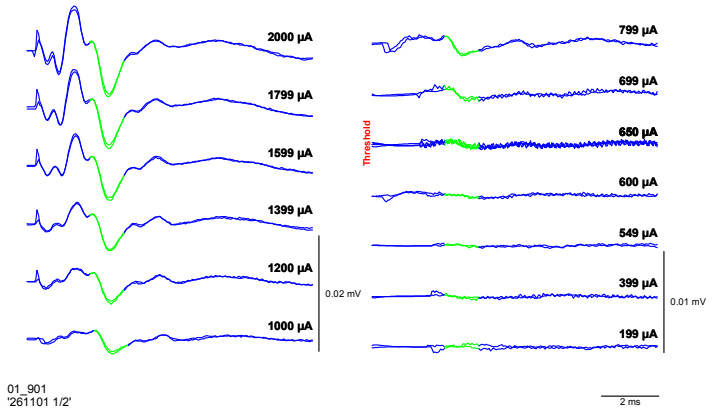
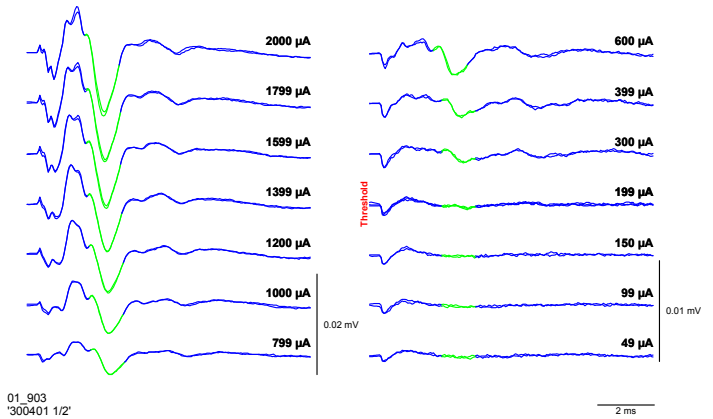


Figure 5. EABRs recorded from NDC_1 immediately following surgery (top panel), and near completion of the stimulation program seven months later (bottom panel). All EABRs were evoked using a 100 µs biphasic current pulse delivered to bipolar electrode pair 1/2. Two responses are typically recorded at each current level. Wave IV, from which response amplitude and latency data are derived, is highlighted in green (light gray if printed in black and white). Threshold is also indicated in each panel.



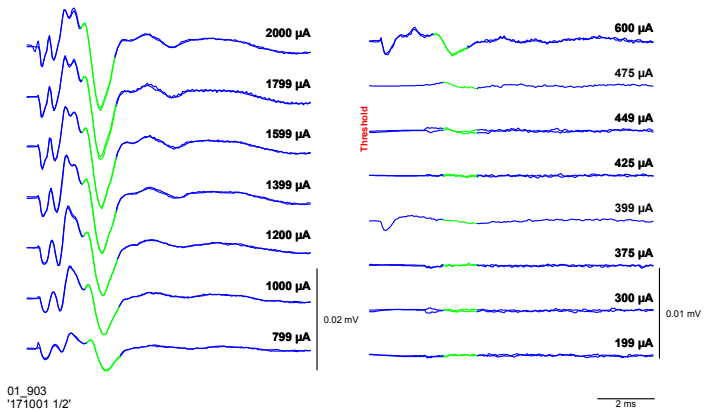
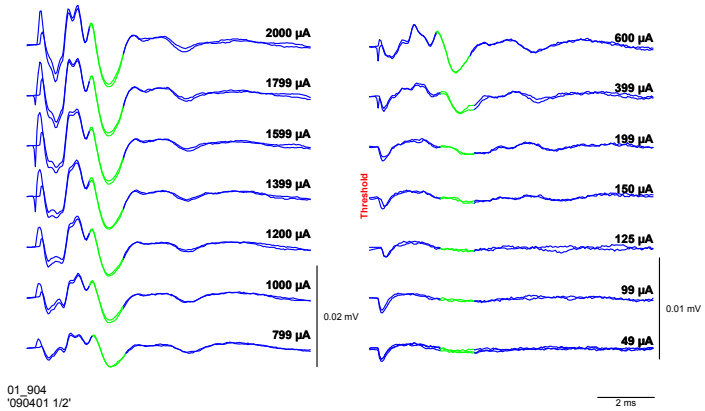


Figure 6. EABRs recorded from NDC_3 immediately following surgery (top panel), and near completion of the stimulation program six months later (bottom panel). These responses were evoked by bipolar pair1/2.



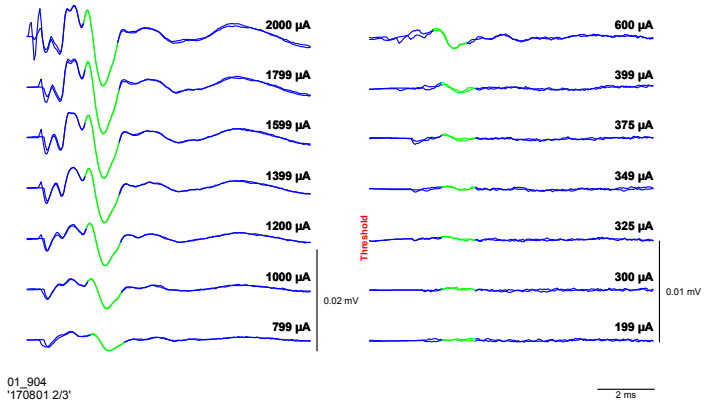
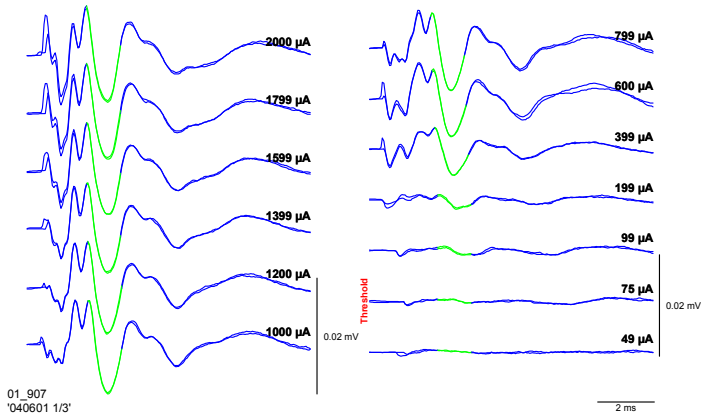


Figure 7. EABRs recorded from NDC_4 immediately following surgery (top panel), and near completion of the stimulation program four months later (bottom panel). Responses in the top panel were evoked by electrode pair 1/2. The bottom panel shows responses evoked from bipolar pair 2/3 as electrode 1 failed during the course of the chronic stimulation program.



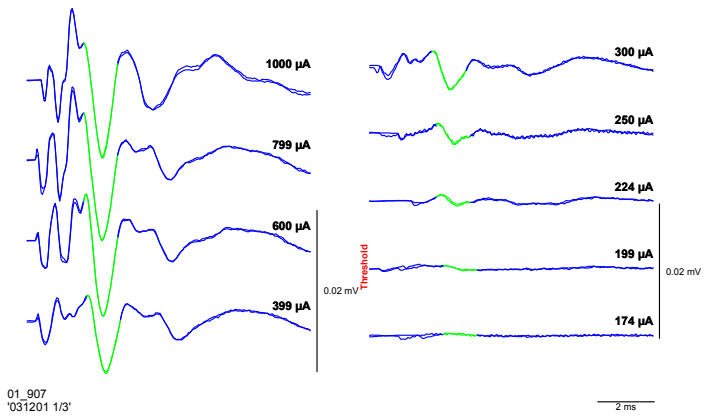


Figure 8. EABRs recorded from NDC_7 immediately following surgery (top panel), and near completion of the stimulation program six months later (bottom panel). These responses were evoked by electrode pair 1/3. The larger inter-electrode spacing resulted in reduced thresholds and a more rapid increase in response amplitude with stimulus intensity compared with electrode pair 1/2 (above).

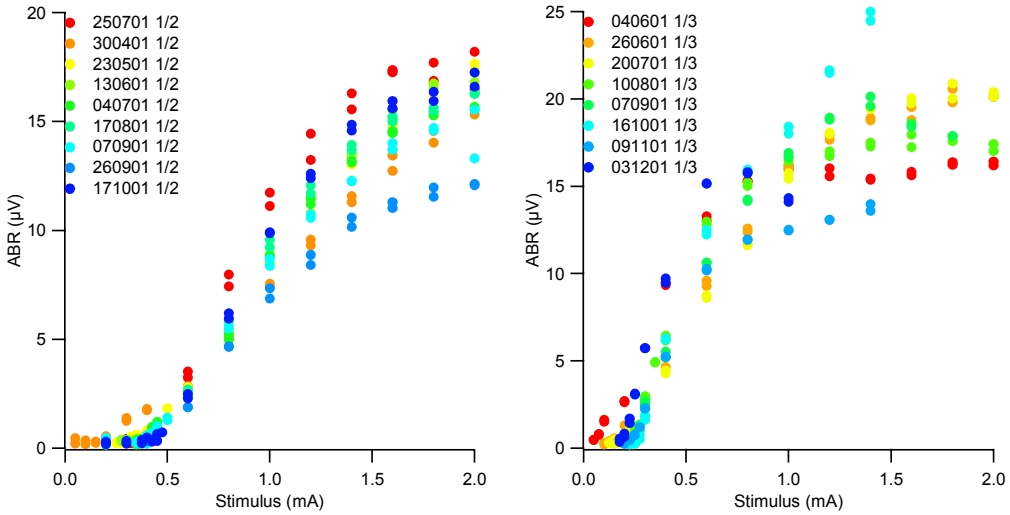


Figure 9. Wave IV EABR i/o functions recorded over the implant period for cat NDC_3 electrode pair 1/2 (left panel), and cat NDC_7 pair 1/3 (right panel). While these i/o curves are relatively stable over the course of the stimulation program, we typically observed a small increase in response threshold over time. Two data points are plotted for each recording session; these reflect the response amplitude from each individual recording.

3.5 Graphic reconstruction of the cochlea

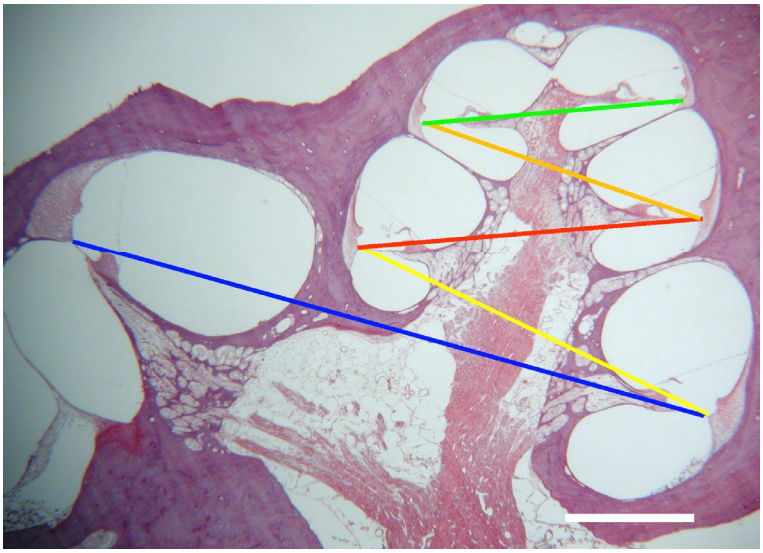


Figure 10. Low power micrograph of a mid-modiolar section of cochlea NDC_3R illustrating the inter-basilar membrane measurements taken in order to graphically reconstruct each cochlea. Each colored line represents a single measure (e.g. lower basal-upper basal (blue); upper basal – lower middle (yellow) etc.). Scale bar =1 mm.

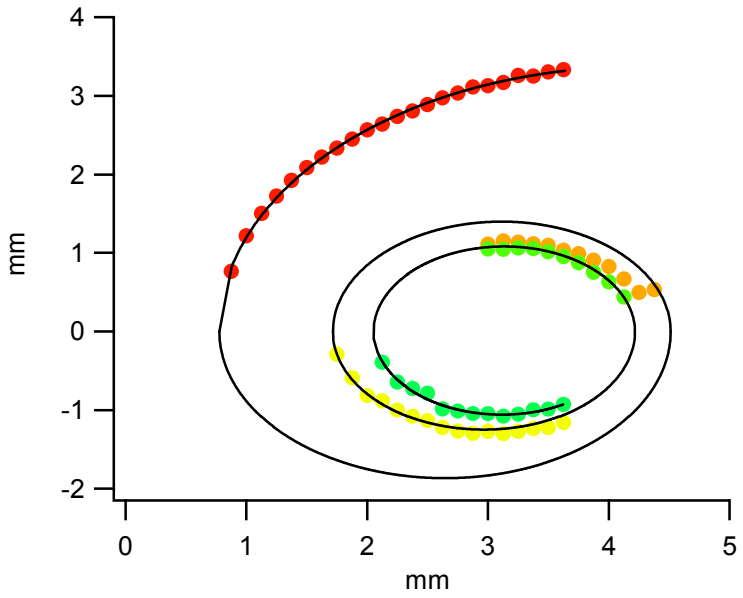


Figure 11. Graphic reconstruction of cochlea NDC_3L. The inter-basilar membrane distance is measured under the microscope; these data are then fitted using a simple arc to give the total basilar membrane length.

The basilar membrane data is fitted with a smooth curve based on the techniques of Schuknecht (1953). The distance from the round window to the basilar membrane in each section is then calculated and displayed (see Table 3).

The curve fitting is based on a simple arc with a function given by:

$$y=ry*sqrt(rx^2 – (x-cx)^2)$$

where cx is the horizontal center of the ellipse described by the arc, rx is the horizontal radius and ry is associated with the eccentricity of the ellipse.

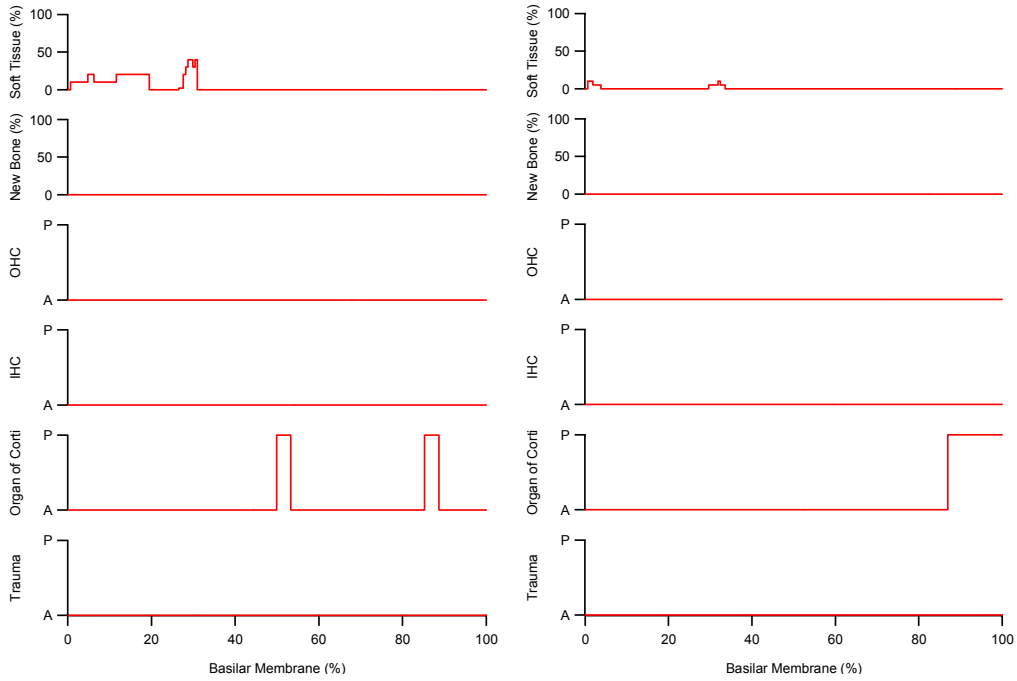
3.6 Cochlear histopathology

In this report we present cochleograms of each of the eight cochleae examined to date; representative photomicrographs illustrating cochlear histology; and finally present the spiral ganglion cell densities for these four animals. Spiral ganglion cell densities in the stimulated cochlea are compared statistically with cell densities measured from the same cochlear region in the contralateral control cochlea.

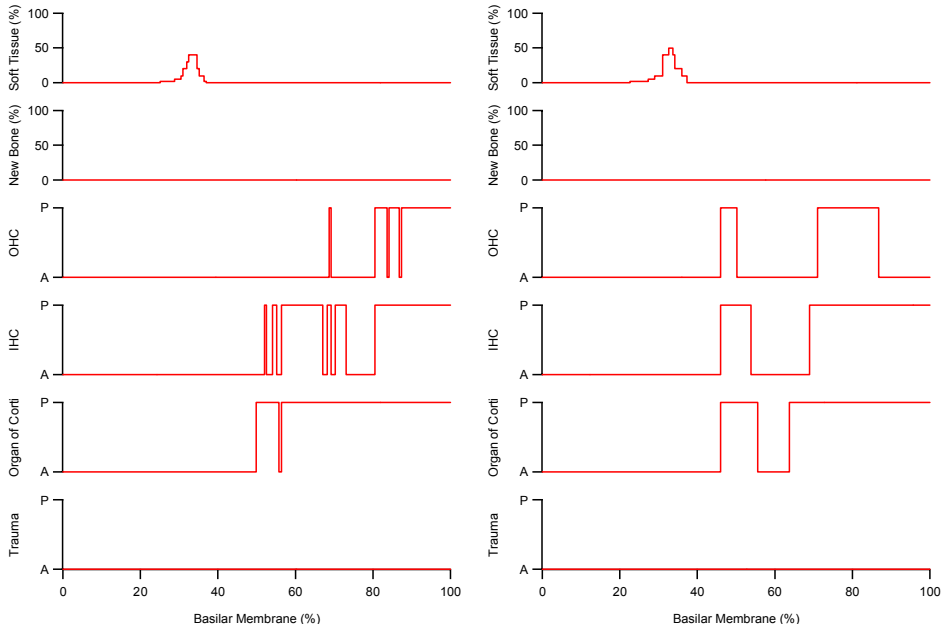
Table 3. Example of graphic reconstruction illustrating cochlea NDC_3L.

Section number (μm)	Distance of basilar membrane from cochlear base (mm)					
	LBT	**UBT**	**LMT**	**UMT**	**LAT**	**UAT**
Section 1500:	2.536	6.461				
Section 1625:	2.342	6.615				
Section 1750:	2.160	6.760	14.709	15.305		
Section 1875:	1.987	6.900	14.343	15.651		
Section 2000:	1.822	7.035	14.109	15.874		
Section 2125:	1.663	7.167	13.919	16.055	21.943	22.731
Section 2250:	1.510	7.296	13.752	16.215	21.677	22.995
Section 2375:	1.360	7.423	13.599	16.362	21.483	23.186
Section 2500:	1.215	7.548	13.456	16.500	21.319	23.349
Section 2625:	1.073	7.674	13.320	16.633	21.172	23.495
Section 2750:	0.934	7.799	13.188	16.761	21.034	23.632
Section 2875:	0.797	7.924	13.060	16.887	20.903	23.762
Section 3000:	0.663	8.051	12.934	17.012	20.776	23.889
Section 3125:	0.530	8.179	12.809	17.137	20.651	24.014
Section 3250:	0.399	8.310	12.684	17.264	20.525	24.140
Section 3375:	0.270	8.444	12.558	17.395	20.399	24.267
Section 3500:	0.141	8.582	12.429	17.530	20.268	**24.398**
Section 3625:	**0.014**	8.725	12.297	17.672	20.132	
Section 3750:	8.876	12.160	17.825	19.986		
Section 3875:	9.037	12.015	17.996	19.826		
Section 4000:	9.210	11.860	18.195	19.638		
Section 4125:	9.403	11.690	18.461	19.390		
Section 4250:	9.626	11.494				
Section 4375:	9.908	11.247				
Section 4500:	10.421	10.802				

Note: The highlighted cells represent the most basal and apical points of the basilar membrane. The total length of the basilar membrane in this example is 24.4 mm (the basilar membrane length in the cat typically varies from ~21.5-24.5 mm). LBT, lower basal turn; UBT, upper basal turn; LMT, lower middle turn; UMT, upper middle turn; LAT, lower apical turn; UAT, upper apical turn.



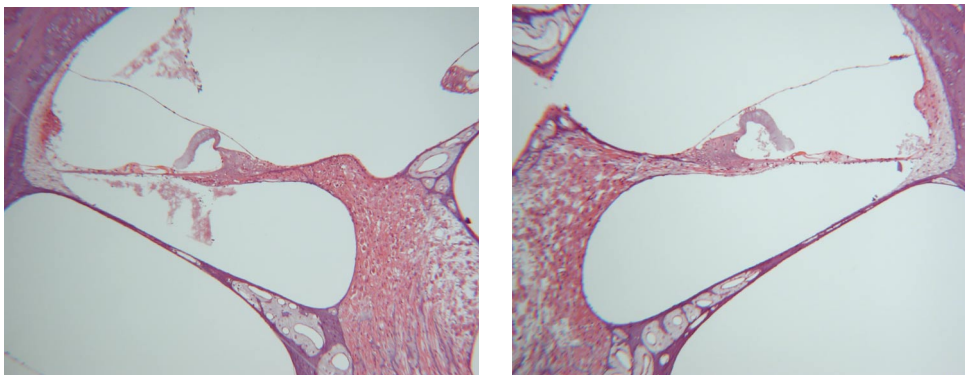
NDC_1L **NDC_1R**



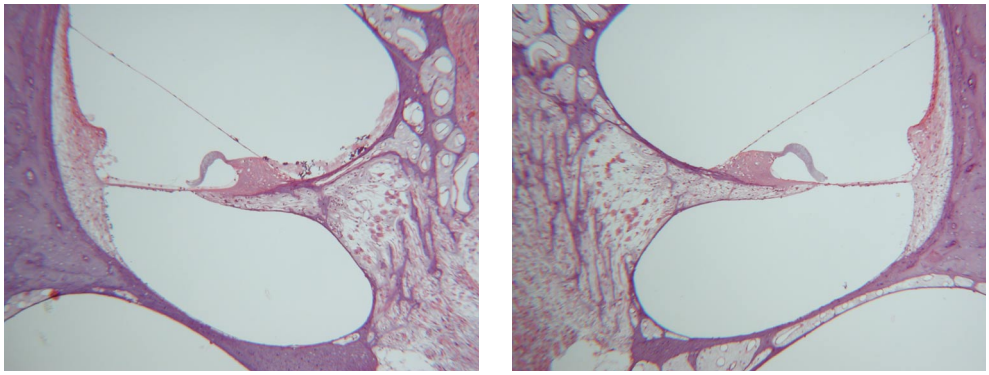
NDC_3L **NDC_3R**

Figure 12. Cochleograms of the stimulated (left) and control (right) cochleae of NDC_1 (top panels) and NDC_3 (bottom panels), illustrating the extent of soft tissue and new bone within the scala tympani (percent estimate); the extent of inner and outer hair cell survival (absent/present); the presence or absence of the structure of the organ of Corti; and the location of any electrode insertion trauma. These data are plotted as a function of distance along the basilar membrane (cochlear base = 0%).

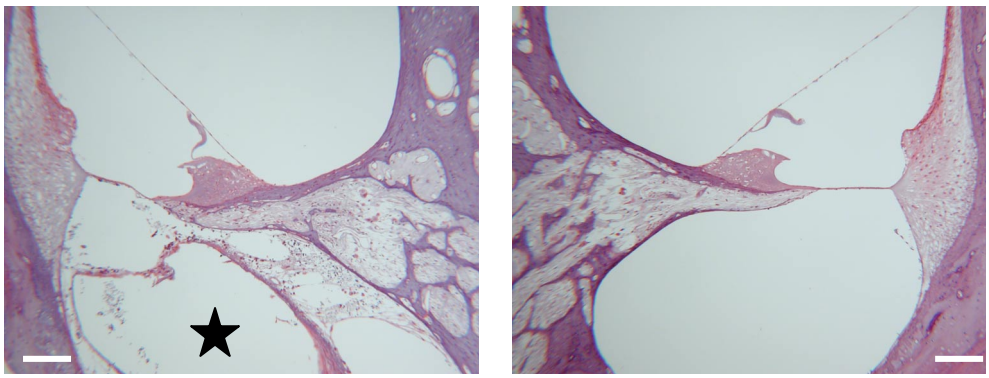
Apical turn



Middle turn



Basal turn



Left (stimulated) Right (control)

Figure 13. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted un-stimulated control (right) cochlea from cat NDC_1. Note that a fibrous tissue capsule associated with the electrode array (star) is often present in the basal turn (the electrode array is removed prior to histology). See Table 2 for duration of deafness and implantation details. Bar = 100 μm.

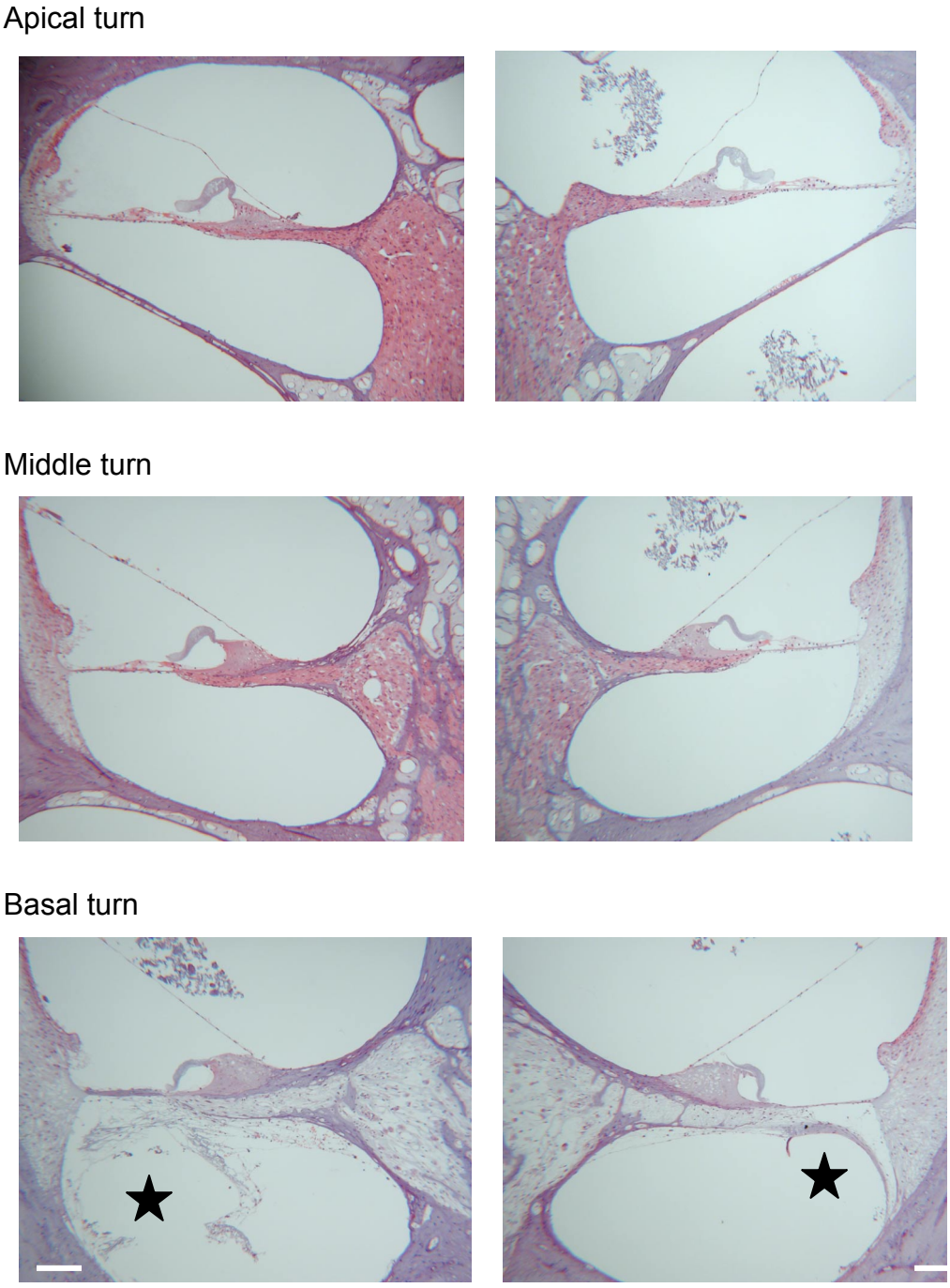
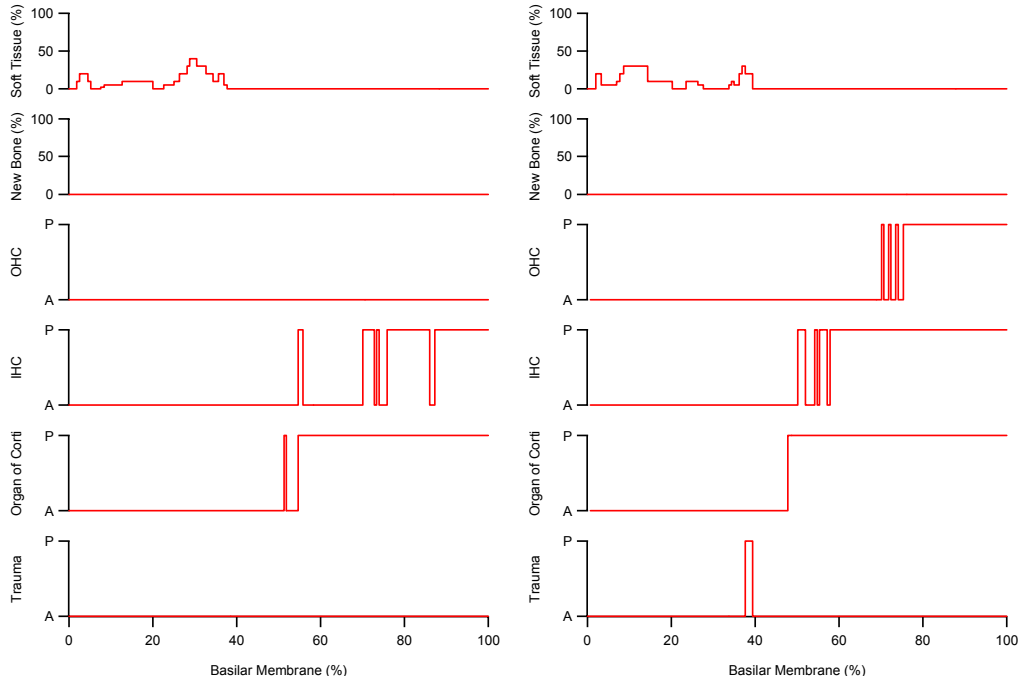
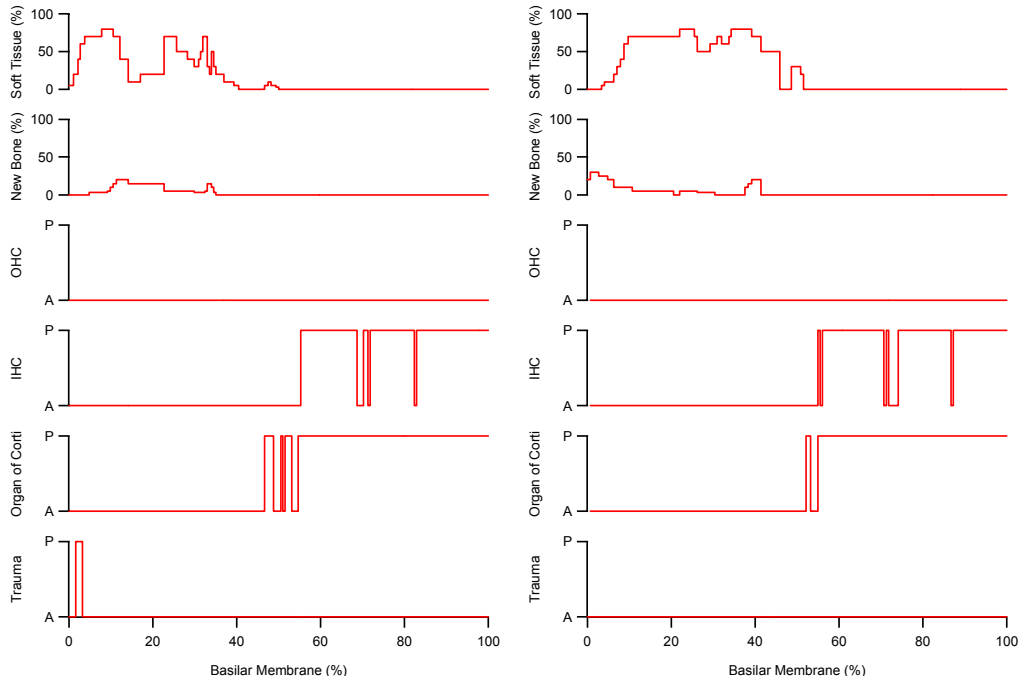


Figure 14. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted un-stimulated control (right) cochlea from cat NDC_3. Some sections show bone dust in the scala vestibuli; this is a preparation artifact. See Table 2 for duration of deafness and implantation details. Bar = 100 μm.



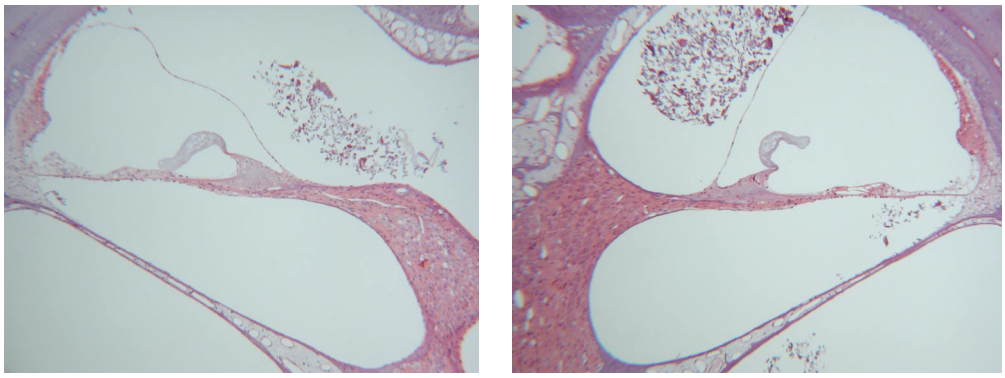
NDC_4L **NDC_4R**



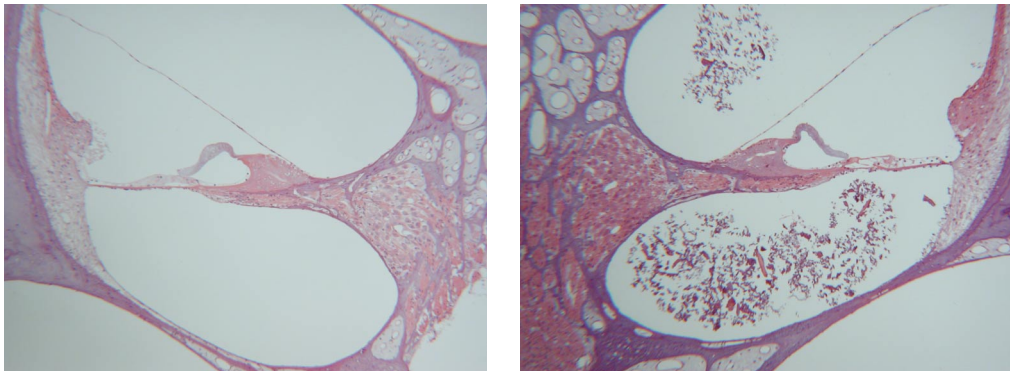
NDC_7L **NDC_7R**

Figure 15. Cochleograms of the stimulated (left) and control (right) cochlea of NDC_4 (top panels) and NDC_7 (bottom panels), illustrating the extent of soft tissue and new bone within the scala tympani (percent estimate); the extent of inner and outer hair cell survival (absent/present); the presence or absence of the structure of the organ of Corti; and the location of any electrode insertion trauma.

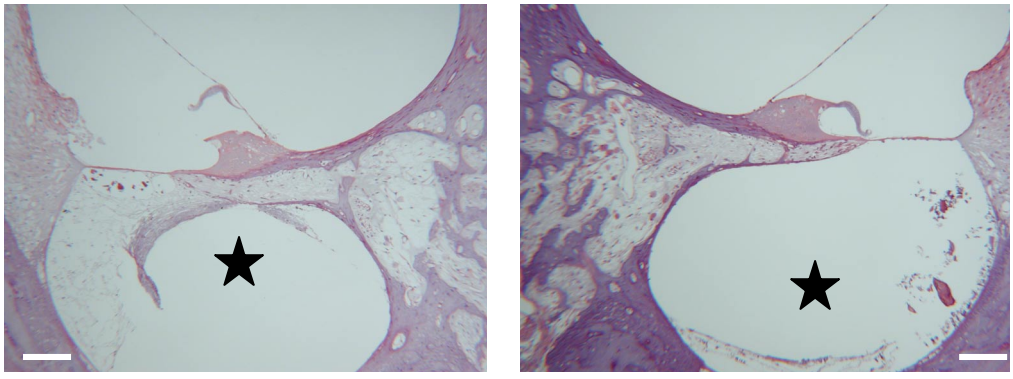
Apical turn



Middle turn



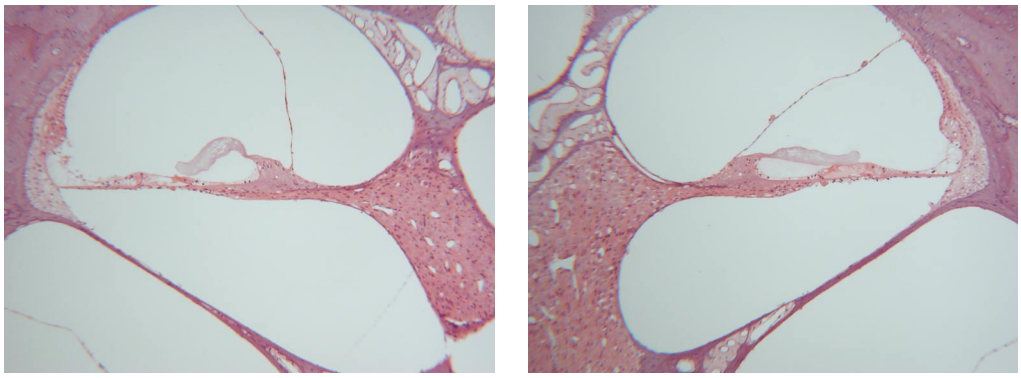
Basal turn



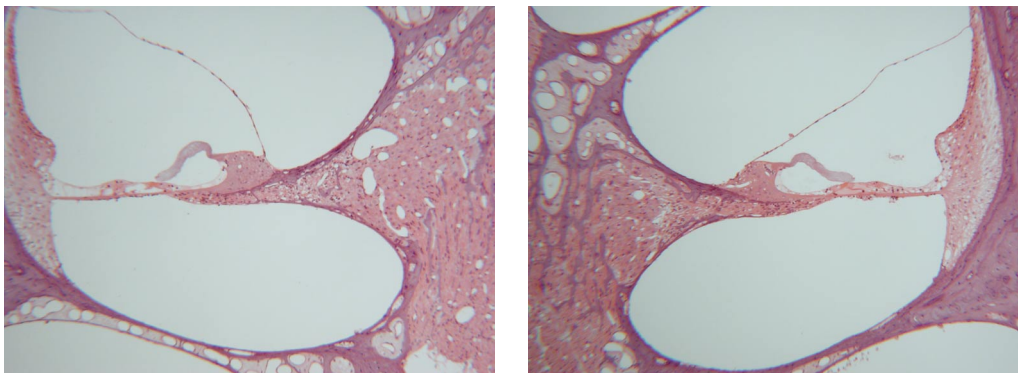
Left (stimulated) Right (control)

Figure 16. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted un-stimulated control (right) cochlea from cat NDC_4. Some sections show bone dust in the scala vestibuli and scala tympani; this is a preparation artifact. See Table 2 for duration of deafness and implantation details. Bar = 100 μm.

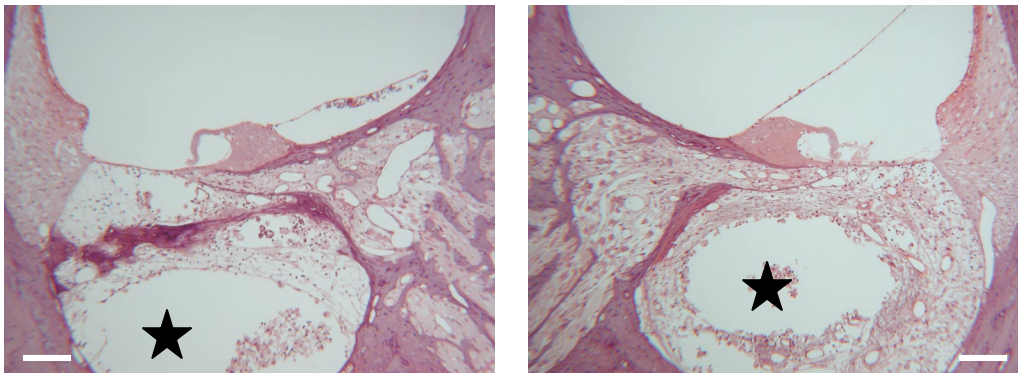
Apical turn



Middle turn



Basal turn



Left (stimulated) Right (control)

Figure 17. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted un-stimulated control (right) cochlea from cat NDC_7. See Table 2 for duration of deafness and implantation details. Note that the more extensive fibrous tissue reaction in NDC_7L is associated with a slightly greater electrode impedance measure compared with the other animals examined (Fig. 2). Bar = 100 μm.

3.7 Spiral ganglion cell densities

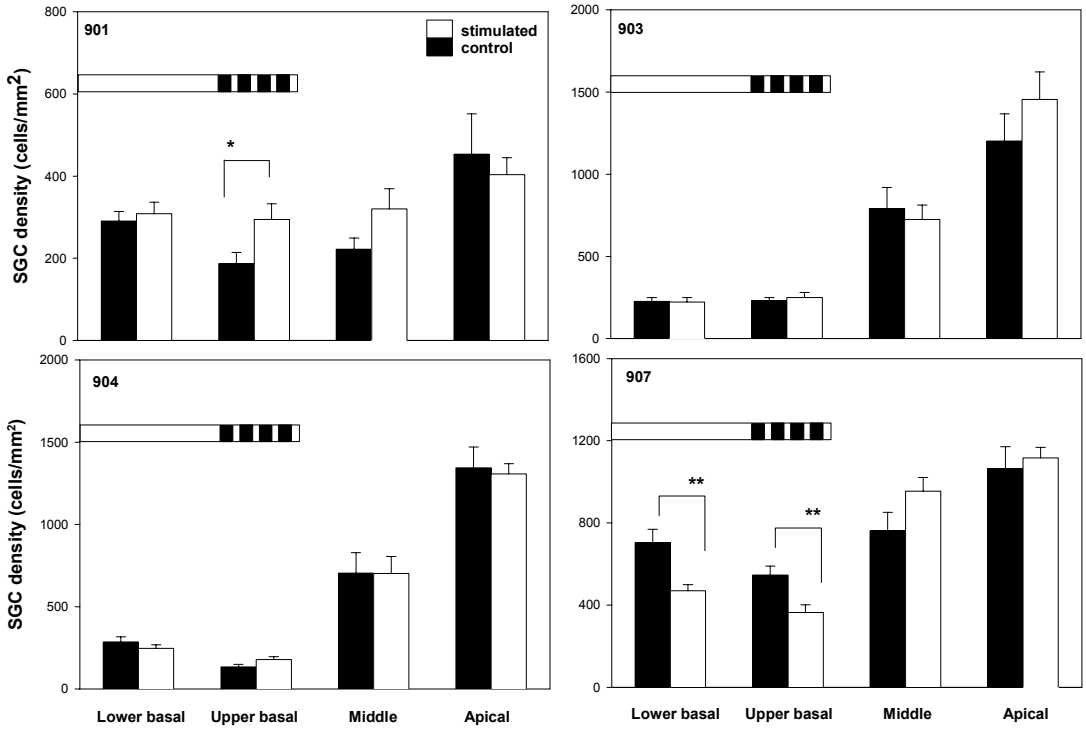


Figure 18. Mean spiral ganglion cell density for the four chronically stimulated animals completed to date. The ganglion cell density measurements have been divided into four cochlear regions; lower basal turn; the upper basal turn (adjacent to the stimulating electrode array); the middle, and apical turns. Stimulated (open) versus control (solid) cell density measures from each animal are compared statistically (t-test). Bar = 1 SEM. *, P<0.05; **, P<0.01.

3.8 Conclusions

We emphasize that the present data are preliminary; we have another 12 chronically stimulated and eight control animals to add to the data pool before our analysis is complete. We anticipate this work will be finalized by the 11th quarter.

The major objective of the present report was to outline the chronic stimulation and monitoring program and the approach used to evaluate the cochlear histopathology.

The use of a severely deaf cohort of experimental animals was designed to model severely deaf cochlear implant subjects. The maintenance of low levels of residual hearing following long-term implantation and electrical stimulation, described in the present report, is consistent with previous experimental (e.g. Xu et al., 1997) and clinical (von Ilberg et al., 1999) studies.

The inclusion of animals with some intact hair cells also allows us to test the hypothesis that residual elements of the organ of Corti play a key role in the trophic support of stimulated spiral ganglion neurons. This follows from the original work of Lousteau, (1987) who reported significant preservation of the spiral ganglion *only* in regions of cochleae exhibiting residual elements of the organ of Corti.

Although limited to a subset of the overall data, we have evidence of relatively stable EABR i/o functions over the course of the stimulation program. Nevertheless, there is a clear trend for a slight increase in EABR thresholds over time. Statistical analysis will be performed and described in a future report. We would argue that an increase in threshold reflects ongoing *loss* of spiral ganglion cells as a result of the deafening process. We have previously reported significant increases in EABR thresholds in deafened animals compared to controls, with greater threshold increases evident in animals deafened for longer periods of time (Hardie and Shepherd, 1999).

A central theme of the present contract is the evaluation of protective effects of chronic electrical stimulation on spiral ganglion cells in deafened cochleae. Our data – limited to four animals at this point – shows no clear evidence of enhanced ganglion cell survival in the stimulated compared with control cochleae. We emphasize that these observations are preliminary; a detailed analysis awaits further data collection, which is currently in progress.

4. Publications

During the quarter the following papers, funded in part or fully by this contract, were accepted for publication:

Shepherd R.K., & Xu, J. A multichannel scala tympani electrode array incorporating a drug delivery system for chronic intracochlear infusion. Hearing Research (*in press*).

Hellier, W.P.L., Wagstaff, S.A., O'Leary, S.J. & Shepherd, R.K. Functional and morphological response of the stria vascularis following a sensorineural hearing loss. Hearing Research (*in press*).

5. Plans for Next Quarter

- Continue our chronic stimulation studies in guinea pigs and long-term deafening studies in the rat.
- Continue the manufacture of guinea pig electrode assemblies.
- Continue histological preparation and analysis of cochleae and auditory brainstem structures in cats and guinea pigs following completion of the chronic stimulation program.
- Continue developing our immunochemistry protocols.

- Continue preparation for manuscript submission and conference presentations.

6. Acknowledgements

We gratefully acknowledge the important contributions made by our Veterinarian Dr Sue Pierce, Elisa Borg for management of our animal house, Helen Feng for electrode manufacture and Rodney Millard and Frank Nielsen for engineering support.

7. References

Hardie, N. A., Shepherd, R. K., 1999. Sensorineural hearing loss during development: morphological and physiological response of the cochlea and auditory brainstem. Hear Res. 128, 147-165.

Huang, C. Q., Shepherd, R. K., Carter, P. M., Seligman, P. M., Tabor, B., 1999. Electrical stimulation of the auditory nerve: direct current measurement in vivo. IEEE Trans Biomed Eng. 46, 461-470.

Lousteau, R. J., 1987. Increased spiral ganglion cell survival in electrically stimulated, deafened guinea pig cochleae. Laryngoscope. 97, 836-842

Schuknecht, H. F., 1953. Techniques for study of cochlear function and pathology in experimental animals. Archives of Otolaryngology. 58, 377-400

von Ilberg, C., Kiefer, J., Tillein, J., Pfenningdorff, T., Hartmann, R., Sturzebecher, E., Klinke, R., 1999. Electric-acoustic stimulation of the auditory system. New technology for severe hearing loss. ORL J Otorhinolaryngol Relat Spec. 61, 334-340.

Xu, J., Shepherd, R. K., Millard, R. E., Clark, G. M., 1997. Chronic electrical stimulation of the auditory nerve at high stimulus rates: a physiological and histopathological study. Hear. Res. 105, 1-29