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Chronic electrical stimulation of the auditory nerve at high stimulus rates: a physiological and histopathological study

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Abstract

A major factor associated with recent improvements in the clinical performance of cochlear implant patients has been the development of speech-processing strategies based on high stimulation rates. While these processing strategies show clear clinical advantage, we know little of their long-term safety implications. The present study was designed to evaluate the physiological and histopathological effects of long-term intracochlear electrical stimulation using these high rates. Thirteen normal-hearing adult cats were bilaterally implanted with scala tympani electrode arrays and unilaterally stimulated for periods of up to 2100 h using either two pairs of bipolar or three monopolar stimulating electrodes. Stimuli consisted of short duration (25–50 μs/phase) charge-balanced biphasic current pulses presented at 1000 pulses per second (pps) per channel for monopolar stimulation, and 2000 pps/channel for bipolar stimulation. The electrodes were shorted between current pulses to minimize any residual direct current, and the pulse trains were presented using a 50% duty cycle (500 ms on; 500 ms off) in order to simulate speech. Both acoustic (ABR) and electrical (EABR) auditory brainstem responses were recorded periodically during the chronic stimulation program. All cochleas showed an increase in the click-evoked ABR threshold following implant surgery; however, recovery to near-normal levels occurred in approximately half of the stimulated cochleas 1 month post-operatively. The use of frequency-specific stimuli indicated that the most extensive hearing loss generally occurred in the high-frequency basal region of the cochlea (12 and 24 kHz) adjacent to the stimulating electrode. However, thresholds at lower frequencies (2, 4 and 8 kHz), appeared at near-normal levels despite long-term electrode implantation and electrical stimulation. Our longitudinal EABR results showed a statistically significant increase in threshold in nearly 40% of the chronically stimulated electrodes evaluated; however, the gradient of the EABR input/output (I/O) function (evoked potential response amplitude versus stimulus current) generally remained quite stable throughout the chronic stimulation period. Histopathological examination of the cochleas showed no statistically significant difference in ganglion cell densities between cochleas using monopolar and bipolar electrode configurations ($P = 0.67$), and no evidence of cochlear damage caused by high-rate electrical stimulation when compared with control cochleas. Indeed, there was no statistically significant relationship between spiral ganglion cell density and electrical stimulation ($P = 0.459$), or between the extent of loss of inner (IHC, $P = 0.86$) or outer (OHC, $P = 0.30$) hair cells and electrical stimulation. Spiral ganglion cell loss was, however, influenced by the degree of inflammation ($P = 0.016$) and electrode insertion trauma. These histopathological findings were consistent with the physiological data. Finally, electrode impedance, measured at completion of the chronic stimulation program, showed close correlation with the degree of tissue response adjacent to the electrode array. These results indicated that chronic intracochlear electrical stimulation, using carefully controlled charge-balanced biphasic current pulses at stimulus rates of up to 2000 pps/channel, does not appear to adversely affect residual auditory nerve elements or the cochlea in general. This study provides an important basis for the safe application of improved speech-processing strategies based on high-rate electrical stimulation.

Keywords: Cochlear implant; High stimulus rate; Electrical stimulation; Cochlear physiology; Auditory brainstem response; Electrically-evoked auditory brainstem response; Electrode impedance; Cochlear histopathology

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1. Introduction

Previous studies using the Melbourne/Cochlear mul-

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tiple channel cochlear implant have shown that chronic electrical stimulation using charge-balanced biphasic current pulses at charge densities in the range 20–52 $\mu C\ cm^{-2}$ geom. per phase and stimulus rates of up to 500 pulses per second (pps) do not adversely affect cochlear structures including auditory nerve fibres (Shepherd et al., 1983, 1990a, 1994a; Clark et al., 1991; Ni et al., 1992). Similar conclusions have been drawn from other studies (Walsh and Leake-Jones, 1982; Leake et al., 1990).

Recently, there has been considerable interest in the development of speech-processing strategies based on high pulse rates. Some psychophysical studies have suggested that new speech-processing strategies based on high stimulus rates (>500 pps) may result in a significant improvement in speech perception (Wilson et al., 1991, 1993; Tong et al., 1990; Dillier et al., 1992; McDermott et al., 1992; Shannon, 1992; Busby et al., 1993). However, before speech-processing strategies based on high rates can be widely applied clinically, it is important to evaluate their safety using an appropriate animal model.

Previous acute experimental studies (e.g., Duckert and Miller, 1982; Miller et al., 1983) have shown that short periods of continuous 1 kHz sinusoidal stimulation in the guinea pig can produce a significant elevation in the electrically-evoked auditory brainstem response (EABR) thresholds. While the thresholds returned to pre-stimulus levels 1 week following the acute stimulus, subsequent histological evaluation of the cochleas showed evidence of pathology. Acute evoked potential studies in our laboratory (Shepherd et al., 1990a; Tykocinski et al., 1995a) have suggested that continuous pulsatile stimulation of the auditory nerve at high stimulus rates and intensities can induce a significant decrement in the amplitude of the electrically evoked auditory brainstem response (EABR). These reductions in amplitude were associated with an increase in both threshold and latency. The extent of these stimulus induced changes were dependent on the extent of evoked neural activity and were, therefore, a function of stimulus rate and stimulus intensity. Not only was stimulus rate a major contributor to the observed reduction in excitability of the auditory nerve, but it also determined the magnitude and the time course of the post-stimulus EABR recovery. Moreover, these stimulus induced changes were observed at the level of wave I of the EABR, implying that they occurred within the auditory nerve. Similar stimulus-induced reductions in the excitability of the auditory nerve have been reported by Killian et al. (1994) using 100 ms bursts of a 16 kHz electrical sine wave delivered to a monopolar round window electrode. Both studies reported that the degree of stimulus-induced reduction in neural excitability was dependent on the amplitude of the stimulus current, and fatigue effects were reduced with decreasing duty cycle. Finally, Agnew et al. (1989) reported stimulus-induced neural fatigue in cat peroneal nerve following acute electrical stimulation using charge-balanced biphasic current pulses presented at high stimulus rates and intensities. Significantly, these authors reported histological evidence of neural damage with increasing stimulus rate.

Given sufficient stimulus intensity, auditory nerve fibres are capable — at least for short periods of time — of discharging in response to every current pulse at stimulus rates far beyond their normal maximum physiological rate (Moxon, 1971; Hartmann et al., 1984; van den Honert and Stypulkowski, 1984; Javel et al., 1987). A number of neurophysiological studies have suggested that electrical stimulation evoking neural activity at unnaturally high discharge rates could have an adverse effect on the neuron's metabolism, and may lead to neural degeneration (Agnew et al., 1989, 1993; Shepherd et al., 1990a; McCreery et al., 1992; Tykocinski et al., 1995a). Indeed, sustained electrical stimulation of the hippocampus has been used as an experimental model of activity-induced epileptic brain damage (Sloviter, 1983; Olney et al., 1983; Wasterlain and Shirasaka, 1994). Stimulus-induced increases in neural activity results in a concomitant increase in neural metabolism (Sokoloff, 1993), together with an increase in the intracellular calcium concentration (Scharfman and Schwartzkroin, 1989; Knopfel and Gahwiler, 1992). High levels of intracellular calcium associated with activity-induced metabolic stress, has been implicated in cell death (Meldrum, 1983; Siesjo et al., 1989; Wasterlain et al., 1993), and may have contributed to the stimulus-induced changes observed in the acute stimulation studies cited above.

However, a potential criticism of these previous acute studies is the use of stimulus intensities well above levels that would be tolerated by the awake animal. We postulate that while stimulation at high rates and intensities may be capable of overcoming relative refractory effects to evoke spike rates well above normal levels, intensities in the range used clinically are likely to evoke near-normal neural activity, and may not, therefore, result in stimulus-induced neuronal damage. For this reason it is important to complement the acute stimulation studies performed in anaesthetised animals with long-term studies in the awake animal, using stimulus paradigms that are more closely related to those used clinically.

Clearly, the application of auditory prostheses using speech-processing strategies based on high stimulation rates requires careful experimental evaluation prior to their extensive clinical application. The major objective of the present study was to evaluate the safety of chronic intracochlear electrical stimulation using charge-balanced biphasic current pulses delivered to monopolar or bipolar scala tympani electrodes operat-

ing at rates of up to 2000 pps/channel. Monopolar and bipolar electrodes were evaluated as both electrode geometries have clinical application. Moreover, given the differences in both threshold and current distribution associated with these two types of electrodes, it was felt that a variation in pathophysiological response may be observed. Some aspects of this work have previously appeared in abstract form (Shepherd et al., 1994b,c)

2. Materials and methods

2.1. Experimental animals and preparation

Thirteen normal-hearing adult cats (26 cochleas) were used in this study. Prior to surgery each animal was examined to ensure that it had otoscopically normal tympanic membranes, and normal-hearing thresholds to click-evoked auditory brainstem response (ABR; <37 dB peak equivalent SPL re 20 μPa; see Section 2.4).

2.2. Implant assemblies

The cochlear implant assembly for this study is shown in Fig. 1. The intracochlear electrodes consisted of platinum (Pt) rings of 0.3 mm width on a Silastic MDX-4-4210 carrier. The carrier tapered from a diameter of 0.43 mm at the tip ring to a diameter of 0.53 mm at a non-active Pt ring, 6 mm from the tip. (This was used as a guide to insertion depth during surgery.) The four active Pt electrodes were located near the tip of the array. The geometrical surface area of each electrode was approximately 0.43 mm^2, and the inter-electrode separation was 0.45 mm. A Pt disc electrode (electrode 1 in Fig. 1), with a diameter of 5 mm and a geometrical area of 19.63 mm^2, was included as a remote electrode for monopolar stimulation. Teflon insulated platinum/iridium (90:10) wires connected each Pt electrode to an insulated, multistranded stainless-steel leadwire. The stainless steel leadwire system provided external access to the electrodes for stimulation and impedance measurements (see Section 2.8). The control electrodes were not connected to a leadwire system. Consequently EABRs and electrode impedances were not obtained from control cochleas.

2.3. Surgical procedure

Each animal was bilaterally implanted: one side with a stimulating electrode array and the opposite side with a control array (Table 1). The cochlea receiving the stimulating electrode was randomly selected. Surgery was performed under sterile conditions; each animal was sedated by a mixture of Ketamil (ketamine hydrochloride, 20 $mg \cdot kg^{-1}$) and Rompun (2% xylazine solution, 3.8 $mg \cdot kg^{-1}$), and anaesthesia was maintained at a surgical level using Rompun/Ketamine (1:1) intravenously during surgery as required. A dorso-lateral approach was used to expose the bulla and a hole drilled to expose the round window. Two small holes were drilled near the opening in the bulla as the sites for fixation of the leadwire. The bulla cavity was flushed with antibiotics (Amoxycillin sodium) and the round window membrane was incised at its inferior-posterior part with the tip of a 25-ga needle. The intracochlear electrode array was carefully inserted for a distance of 6 mm along the scala tympani. Following the insertion, the round window was sealed with a small piece of fascia or muscle. The extracochlear Pt disc electrode was placed either inside or just outside the bulla. The leadwire, which was fixed using Dacron mesh ties placed through the two small holes created in the bulla, passed subcutaneously towards the skull and fixed to the parietal bone close to the nuchal crest using an additional Dacron mesh tie. The leadwire was then passed subcutaneously to exit the skin via a small incision in the neck.

Systemic antibiotics (Cloxacillin and Duplocillin) were administered following each surgical procedure. During surgery the animal's temperature was monitored by a rectal probe and maintained at 38 ± 1°C, respiratory rate and expired CO_2 level were monitored (NORMOCAP 200) and maintained within normal clinical levels (respiration rate: 8–18/min; expired CO_2 $<5\%$).

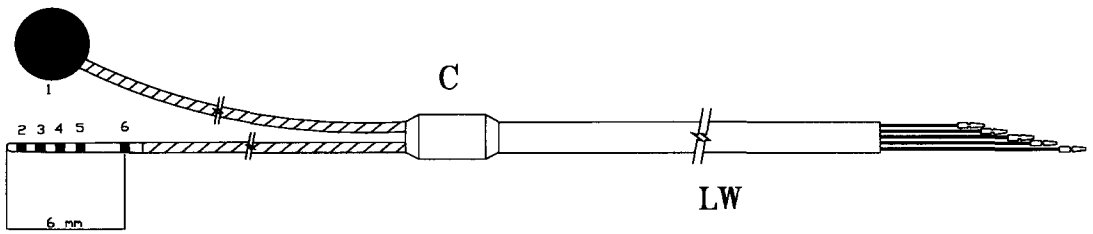

Fig. 1. Cochlear implant assembly used in this study. (1) Extracochlear Pt disc electrode (used in monopolar stimulation); (2–5) Pt band intracochlear electrodes; (6) non-active Pt ring used as a guide to insertion depth. C, Connector; LW, stainless steel leadwire system. The electrode numbers used throughout this paper are as indicated in this figure.

2.4. Auditory brainstem responses

ABRs to click stimuli were recorded periodically during the chronic stimulation program (prior to and 7–10 days following surgery, and every 300 h of stimulation) to monitor the hearing status of both ears of each animal. All recordings were made in a sound-attenuated, electrically shielded room with the animal sedated by a mixture of ketamine hydrochloride (20 $mg \cdot kg^{-1}$) and xylazine (3.8 $mg \cdot kg^{-1}$). Computer generated 100 μs duration, rarefaction clicks were presented from a Richard Allen DT-20 loudspeaker placed 10 cm from the ipsilateral pinna. The contralateral external ear canal was blocked with an ear mold compound (Otoform). ABRs were recorded using subcutaneous needle electrodes (vertex +ve, neck −ve, thorax ground). The responses were amplified by a factor of 10^5, and filtered by a bandpass filter (high-pass: 150 Hz, 24 dB/octave; low-pass: 3 kHz, 6 dB/octave; Krohn-Hite 3750R). The output of the amplifier was fed to a 10-bit A/D converter and sampled at 20 kHz for a period of 12.5 ms following the click onset and 500 responses were averaged and stored for subsequent analysis. Two recordings were made at each stimulus level. An attenuator was used to reduce the intensity in order to establish threshold. Threshold was defined as the lowest stimulus level for which wave IV ($\approx$4.0–4.5 ms latency relative to the stimulus onset) was at least 0.2 μV in both recordings. During the recording, the animal's body temperature was maintained at 38 ± 1°C.

2.5. Compound action potentials

Acoustically evoked compound action potentials (CAP) were recorded periodically in all cochleas implanted with a stimulating electrode in order to establish the frequency-specific nature of the hearing loss. The acoustic stimuli consisted of tone-pips (1 ms rise/fall time; 3 ms plateau) at frequencies of 2, 4, 8, 12 and 24 kHz. Tones were generated by a Datapulse 410 function generator and amplitude modulated using an HP 8010A pulse generator. The tone-pips were presented from a Richard Allen DT-20 loudspeaker placed 10 cm from the ipsilateral pinna. CAPs were recorded differentially using the most apical scala tympani stimulating electrode (+ve) against a subcutaneous needle (neck −ve; thorax ground). The responses were amplified by a factor of 10^4, while the transducer and filtering were the same as that used for ABRs. CAP threshold for each frequency tested was taken as the lowest stimulus level in which the response was visually detected using a Tektronics 420 cathode ray oscilloscope (Rajan et al., 1991).

2.6. Electrically-evoked auditory brainstem responses

EABRs were recorded prior to and following every 300 h of the chronic stimulation program in order to monitor changes in the threshold and the suprathreshold response of the auditory nerve. The anaesthetic regime has been described above (Section 2.4). Electrical stimuli were generated from an optically isolated, charge-balanced biphasic current source with variable current amplitude, pulse width and repetition rate. The pulse width used to evoke the EABRs was set at 25 μs/phase for the monopolar stimulated cochleas and 50 μs/phase for those cochleas stimulated using a bipolar electrode configuration. These pulse widths were same as those used during the chronic stimulation program (see Section 2.7). A repetition rate of 33 pps was used for all EABR recordings. The EABR recording system was identical to that used for ABRs except that an artifact suppressor was used to minimize the stimulus artifact. This was achieved by using a sample-and-hold circuit prior to the bandpass filter, in which the amplifier output was sampled just before the stimulus onset and held at that level for the duration of the stimulus. If present, electrophonic activity — illustrated by longer response latencies (e.g., wave IV $>$3.5 ms) — was masked by continuous white noise (WN) produced by a 8057A Hewlett-Packard precision noise generator (Black et al., 1983). The output of the white noise generator was fed to the Richard-Allen DT-20 loudspeaker placed 10 cm from ipsilateral pinna. The WN intensity used was just sufficient to mask the electrophonic response and consequently varied from animal to animal. Two EABRs were recorded at each current level. EABR I/O functions were determined by measuring the mean amplitude (peak to trough) of wave IV ($\approx$2.7–3.4 ms) and plotting this amplitude against stimulus current. Thresholds were taken as the lowest current level for which wave IV was at least 0.2 μV in both recordings.

2.7. Electrical stimulation program

The portable programmable stimulators used in this study were designed in the Department of Otolaryngology, the University of Melbourne and manufactured by Cochlear Ltd. The stimulus regime consisted of charge-balanced biphasic current pulses delivered at (1) 1000 pps/channel to three intracochlear monopolar electrodes or (2) 2000 pps/channel to two intracochlear bipolar electrode pairs. In monopolar configuration the extracochlear Pt disc served as the remote return electrode for all three channels. The pulse widths were fixed at 25 μs/phase for monopolar stimulation and 50 μs/phase for bipolar stimulation. All current pulses included a 7 μs interphase gap. The stimuli were pulsed using a 500 ms on/500 ms off cycle to simulate speech (Fig. 2). Direct

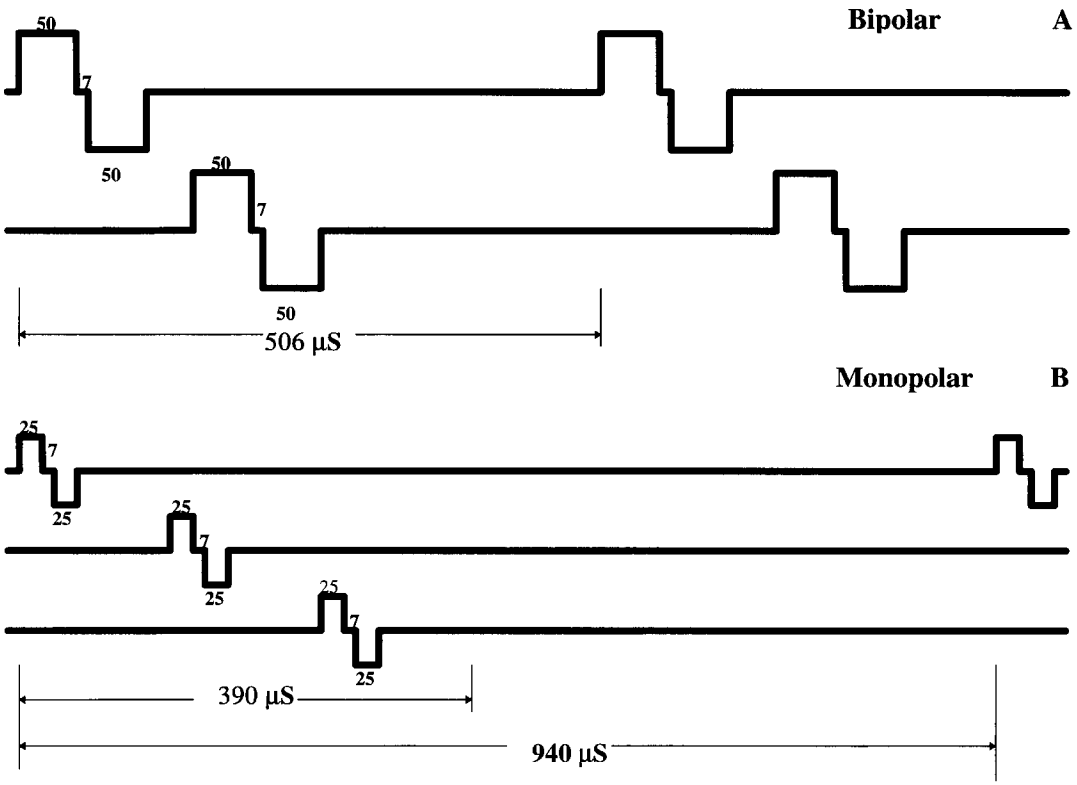

Fig. 2. The stimulus waveforms used in bipolar (A) and monopolar stimulation (B). Note that bipolar electrodes were stimulated at ≈2000 pps/channel (2 channels) while monopolar electrodes were stimulated at ≈1000 pps/channel (3 channels). In both monopolar and bipolar configurations the electrodes were shorted between current pulses to minimize DC (see text).

current (DC) was minimized by electrode shorting between current pulses (Patrick et al., 1990). DC levels were checked for all stimulators in vitro before in vivo use, and showed maximum DC levels of less than 0.1 μA for the stimulus levels used in the present study. These levels of DC are comparable with our previous studies (Shepherd et al., 1983, 1990b, 1994a; Ni et al., 1992). DC levels were monitored by measuring the average voltage developed across a 100 Ω resistor in series with the electrode using an integrating DC voltmeter (DSE Digital Multimeter Q 1550).

Chronic electrical stimulation commenced 8–15 days following surgery. Five cats were stimulated via the bipolar scheme (pair 2/4 and 3/5 or 2/3 and 4/5; Fig. 1), while eight cats were stimulated via the monopolar scheme (pair 1/2, 1/3, 1/4; Fig. 1). During the course of the chronic stimulation program, two of the eight monopolar animals were changed to bipolar stimulation due to electrically evoked facial nerve activity at stimulus levels just above the animals EABR threshold. All cochleas were identified according to the mode of stimulation (see note in Table 1). The stimulus current level for each animal was set midway between the behavioural threshold — determined by changes in heart rate and attentiveness — and a level that evoked an aversive response. This level was confirmed to be suprathreshold for direct electrical excitation of the auditory nerve using EABR recordings. WN masking of electrophonic activity during EABR recordings ensured that the animals were stimulated via direct activation of the auditory nerve and not via electrophonic activity which — particularly for bipolar stimulation — tends to have a lower threshold (Marsh et al., 1981; Black et al., 1983; Shepherd et al., 1983). Stimulus current amplitudes used in the study were in the range 0.4–1.25 mA. Charge densities varied from 4.76 to 14.88 $\mu C \cdot cm^{-2}$ geom. per phase for bipolar stimulation and 2.38–5.65 $\mu C \cdot cm^{-2}$ geom. per phase for monopolar stimulation (Table 1).

The constant-current stimulator was carried in a harness worn by the cat to enable continuous stimulation without confining the animal's activities. The animals received approximately 16 h of electrical stimulation per day for a total stimulation period ranging from 350 to 2137 h. The unstimulated control cochleas were implanted for a similar period to that of the stimulated cochleas (Table 1).

2.8. Electrode impedance

During the long-term implantation period, the stimulus current, electrode voltage waveforms and DC lev-

Table 1
Chronic electrical stimulation at high stimulus rates in normal-hearing cats: summary of animal groups and stimulus parameters

Stimulated cochleas		Mode of stim.[a]	Electrodes stimulated	Stimulus current (mA)	Charge density[b] (μC cm^{-2}/phase)	Stim. hours	Total stim. hours	Control cochleas	
Cochlea[a]	Implant duration (days)							Cochlea[a]	Implant duration (days)
M1	36	M	1/2	0.60	3.57	350	350	MC1	36
			1/3	0.50	2.98				
			1/4	0.60	3.57				
M2	30	M	1/2	0.50	2.98	680	680	MC2	30
			1/3	0.50	2.98				
			1/4	0.50	2.98				
M3	83	M	1/2	0.60	3.57	740	1174	MC3	83
			1/3	0.60	3.57				
			1/4	0.60	3.57				
			1/4	0.60	3.57	434			
			1/5	0.60	3.57				
M4	94	M	1/2	0.50	2.98	1258	1258	MC4	94
			1/3	0.60–0.65	3.57– 3.87				
			1/4	0.60	3.57				
M5	104	M	1/2	0.40–0.52	2.38– 3.10	1618	1618	MC5	104
			1/3	0.60–0.65	3.57– 3.87				
			1/4	0.80–0.83	4.76– 4.94				
M6	127	M	1/2	0.43	2.56	2137	2137	MC6	127
			1/3	0.50	2.98				
			1/4	0.48	2.86				
MB1	71	M	1/2	0.60	3.57	615	1072	MBC1	71
			1/3	0.60	3.57				
			1/4	0.70	4.17				
		B	2/4	0.98	11.67	457			
			3/5	0.95	11.31				
B1	85	B	2/4	0.40	4.76	1222	1222	BC1	85
			3/5	0.48–0.50	5.71– 5.95				
B2	102	B	2/4	0.48–0.60	5.71– 7.14	1619	1619	BC2	102
			3/5	0.90–1.25	10.71–14.88				
B3	106	B	2/4	0.40–0.50	4.76– 5.95	1639	1639	BC3	106
			3/5	0.48–0.69	5.71– 8.21				
B4	134	B	2/4	0.50–0.60	5.95– 7.14	2127	2127	BC4	134
			3/5	0.95–0.98	11.31–11.67				
B5	139	B	2/3	0.45–0.60	5.36– 7.14	2132	2132	BC5	139
			4/5	0.70–1.00	8.33–11.90	1884			
BM1	108	M	1/2	0.75–0.95	4.46– 5.65	503	1603	BMC1	108
			1/3	0.85–0.90	5.06– 5.36				
			1/4	0.85–0.90	5.06– 5.36				
		B	2/4	0.65–0.80	7.74– 9.52	1100			
			3/5	0.80–0.95	9.82–11.31				

[a]Cochleas are named according to the mode of stimulation. M, monopolar; B, bipolar; C, control cochlea.
[b]For monopolar electrodes charge density is based on the geometrical surface area of the scala tympani electrode.

els were monitored twice daily using electrically isolated monitoring equipment to ensure that the appropriate stimulation levels were set for each animal, and to provide an indication of electrode impedance. An equivalent circuit used to describe an electrode in electrolyte has been described previously (Shepherd et al., 1990b). Stimulus current (I) and electrode voltage (V_a and V_p) levels were recorded, and electrode impedance ($Z_e = V_p\, i^{-1}$) and access resistance ($R_a = V_a\, i^{-1}$) were calculated. The impedance of an electrode reflects the electrical status of the electrodes and their adjacent tissue environment (Agnew et al., 1983; Shepherd et al., 1990a). DC levels were maintained at less than 0.1 μA throughout the long-term stimulation period.

2.9. Histological procedure

The histological procedure has been reported previously (Shepherd et al., 1991, 1994a; Ni et al., 1992). Briefly, each animal was deeply anaesthetized (see Section 2.4) and the general condition of the bulla and round window were examined. A swab of the bulla was taken for microbiological examination. After the electrode array was removed from the scala tympani and the oval window was opened, the cochlea was gently perfused with 0.1 M phosphate-buffered (pH = 7.4) solution of 1% paraformaldehyde and 2.5% glutaraldehyde through the round window. The solution was aspirated via the oval window. The animal was then killed

Table 2
Inflammation grade and mean spiral ganglion cell density

Cochlea	Total stimulation time (h)	Inflammation grade[a]	Mean SGC[b] density (cells/mm^2)					
			(lower basal turn)[c]	SE	(upper basal turn)[d]	SE	(middle apical turn)[e]	SE
Stimulated cochleas								
M1	350	V	758.71	62.83	660.19	73.66	898.30	128.50
M2	680	II	1863.11	100.63	1973.88	69.15	1722.44	55.54
M3	1174	II	1621.64	75.84	1539.11	64.54	1626.00	62.40
M4	1258	III	664.89	97.87	1004.82	74.04	1104.38	45.14
M5	1618	II	1760.38	143.56	2077.82	86.03	1884.85	49.46
M6	2137	IV	748.01	47.85	609.31	66.80	1387.92	57.72
MB1	1072	II	1180.25	38.70	1142.71	68.14	1140.19	60.56
B1	1222	II	1212.36	105.39	1386.60	73.36	1520.94	46.57
B2	1619	I	1917.70	121.89	1833.65	88.58	2027.09	36.32
B3	1639	II	1263.74	168.67	1854.92	64.39	1715.56	56.09
B4	2127	II	1587.13	81.95	1282.74	108.27	1561.37	43.69
B5	2132	IV	1127.60	67.73	872.36	98.06	1688.49	65.04
BM1	1603	II	1298.92	67.33	1440.73	42.01	1440.54	42.67
Control cochleas								
MC1	–	I	1564.86	89.88	1681.64	60.64	1477.89	60.23
MC2	–	I	1803.50	96.54	1653.25	65.83	1559.94	56.17
MC3	–	I	1604.91	63.74	1565.03	59.90	1483.85	61.06
MC4	–	V	451.50	36.59	253.53	31.06	428.02	38.14
MC5	–	III	788.87	60.84	755.12	92.92	1597.58	49.46
MC6	–	IV	609.96	54.97	375.22	26.44	563.16	55.29
MBC1	–	II	929.73	41.44	858.06	51.50	1038.23	66.82
BC1	–	I	1362.96	105.88	1560.73	61.33	1597.15	30.72
BC2	–	II	1189.70	102.60	1132.26	61.40	1288.27	53.33
BC3	–	I	1172.64	163.96	1577.39	52.40	1541.91	46.29
BC4	–	I	380.51	57.60	367.38	49.09	995.70	32.77
BC5	–	I	1277.34	175.08	1780.41	98.77	1819.93	55.37
BMC1	–	I	1270.42	128.50	1494.55	82.08	1477.18	35.97

[a]Inflammation grade: I, absent; no evidence of an implanted electrode array. II, electrode tissue capsule with or without a loose fibrous tissue within scala tympani. III, small regions of acute/chronic inflammatory cells within scala tympani. IV, acute/chronic inflammatory cells occupying entire scala tympani. V, acute/chronic inflammatory cells spread into Rosenthal's canal and other cochlear structures.
[b]SGC: spiral ganglion cell.
[c]Lower basal turn: Ganglion cell densities in Rosenthal's canal from round window to the site 2 mm basal to the basal-most electrode ($\approx$0–10% cochlear length).
[d]Upper basal turn: Ganglion cell densities in Rosenthal's canal from a site 2 mm basal to the basal-most electrode to 2 mm apical to the tip electrode.
[e]Middle apical turn: Ganglion cell densities in Rosenthal's canal in the apical region of the cochlear ($>$ 50% cochlear length).
[f]SE: standard error.

with an overdose of anaesthetic (pentobarbitone sodium) and perfused intra-arterially with pre-wash (heparinized normal saline, buffered to a pH of 7.35 with 0.1 M phosphate at 37°C) followed by the fixative (1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer at 4°C). The temporal bones were removed and the cochleas were trimmed, decalcified (4% EDTA and 2.5% glutaraldehyde, buffered to a pH of 7.35 with 0.1 M phosphate buffer), embedded in resin (Spurr) and sectioned at a thickness of 2 μm in the horizontal plane. Sections every 120 μm were collected and stained with hematoxylin and eosin (H and E) or 1% aqueous thionin.

The histopathological assessment of each cochlea was made using an image analyser. All cochlear sections were examined microscopically. The image was recorded by a video camera (Panasonic WV-BL200) and digitized via a Data Translation DT2851 frame-grabber card. Significant histological points or areas could be marked by the cursor and stored on a PC. Parameters which were evaluated for each section included IHC and OHC survival expressed as present (100%) or absent (0%), the presence of supporting cells of the organ of Corti (also expressed as 100% or 0%), an estimate of the percentage survival of the spiral ganglion cells within Rosenthal's canal and their peripheral processes within the osseous spiral lamina (OSL), the presence and extent of any inflammatory response, the location and extent of electrode insertion trauma, and the extent of new bone growth in each scala. A computer-aided 3-dimensional graphic reconstruction of each cochlea was made (Seldon, 1991; Kawano et al., 1996). This made it possible to establish the location of the intracochlear

Fig. 3. A: Click evoked ABR thresholds as a function of implantation time for cochlea B2. This cochlea was chronically stimulated for a period of 1600 h. The initial threshold (0 days) was recorded just prior to implantation. ABR threshold increased immediately following implant surgery; however, it returned to pre-surgical levels by 1 month post-implantation. B: Acoustically evoked CAP thresholds as a function of stimulus frequency monitored longitudinally over the implant period (102 days). While thresholds were at or near normal levels at low frequencies, a moderate hearing loss at 12 kHz was observed. This hearing loss was located in the region of the cochlea close to the apex of the electrode array. Interestingly, near-normal thresholds were observed at 24 kHz. Shading illustrates the mean threshold ±1.96 SD as a function of frequency for normal adult cats (Rajan et al., 1991). Lower thresholds at 2 and 4 kHz observed in the present study presumably reflect differences in the site of the recording electrode (5 mm inside the scala tympani versus an electrode located on the round window). C: Click-evoked ABRs from B2 recorded pre-implantation (left) and following 102 days of cochlear implantation and 1600 h of electrical stimulation (right) showed minimal threshold change. Note, however, that the latency of the ABR was ≈0.5 ms longer than the pre-surgical ABR at near-threshold intensities, indicating a reduction in the high-frequency component of the response. The intensity of the click (in dB p.e. SPL) is shown to the right of each response. Two responses were recorded at each intensity. D: Photomicrograph of the upper basal turn of stimulated cochlea B2 (H and E), illustrating a minimal tissue reaction (arrow) in response to the scala tympani electrode array (Inflammation grade I), and normal hair cell and organ of Corti structure both adjacent and apical to the stimulating electrodes. The spiral ganglion cells (sg) and peripheral processes (p) appeared in normal populations. Bar = 100 μm.

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electrode array and the histopathological changes accurately within the scala tympani. Thus, a cochleogram of the resultant cochlear pathology could be constructed and plotted quantitatively as a function of basilar membrane length expressed in percent from base (0%) to apex (100%). Finally, the degree of inflammation was classified according to the site, extent and nature of the reaction as summarized in Table 2 (Clark and Shepherd, 1984).

2.10. Spiral ganglion cell densities

In addition to an estimate of the percentage survival of the spiral ganglion along the entire cochlear length, spiral ganglion cell densities in Rosenthal's canal were calculated in three specific cochlear locations: (1) in the upper basal turn (from a point 2 mm basal to the most basal electrode to a point 2 mm apical to the most apical stimulating electrode), representing the ganglion cell population that had undergone chronic electrical stimulation; (2) the lower basal turn (approximately the first 10% of the cochlea); and (3) the upper middle/apical turn (the apical half of the cochlea). All ganglion cells containing a nucleus were counted and the total count divided by the area of Rosenthal's canal to give ganglion cell density. (Any capillary or isolated bony structure within Rosenthal's canal with an area greater than 100 μm^2 were not included in the area calculation.) This quantitative measure of ganglion cell survival was used to evaluate the effects of chronic electrical stimulation statistically.

2.11. Statistical analysis

Linear regression analysis was used to evaluate the effects of length of stimulation on EABR threshold and the slope of the I/O function over time for each electrode pair. The slope of the I/O function was determined by $(A_m - A_t)/(I_m - I_t)$; where A_m is the maximum response amplitude in μV; A_t is the response amplitude at threshold; I_m is the stimulus current (in mA) required to evoke A_m; and I_t is the threshold current level. Spiral ganglion cell densities in the upper basal turn for monopolar stimulated cochleas were statistically compared with bipolar stimulated cochleas using the nonparametric Mann-Whitney U test. Spiral ganglion cell densities in each of the three regions of the stimulated cochleas were statistically compared with the corresponding region in the contralateral control cochlea using the paired t-test and the non-parametric Mann-Whitney U test. Kruskal-Wallis ANOVA was used to compare the degree of inflammation in scala tympani with spiral ganglion cell density, duration of implantation and duration of electrical stimulation. The same statistical test was used to compare the population of hair cells (expressed as the mean percentage survival) within the upper basal turn, i.e., adjacent to the electrode array, with the mean percentage survival apical to the array (i.e., in the middle/apical turns). In addition, this test was also used to evaluate the status of IHCs and OHCs and the organ of Corti within the upper basal turn, by comparing their mean percentage survival as a function of electrical stimulation. Finally, the degree of inflammation within the scala tympani was compared statistically with both IHC and OHC survival adjacent and apical to the electrode array using the Kruskal-Wallis ANOVA.

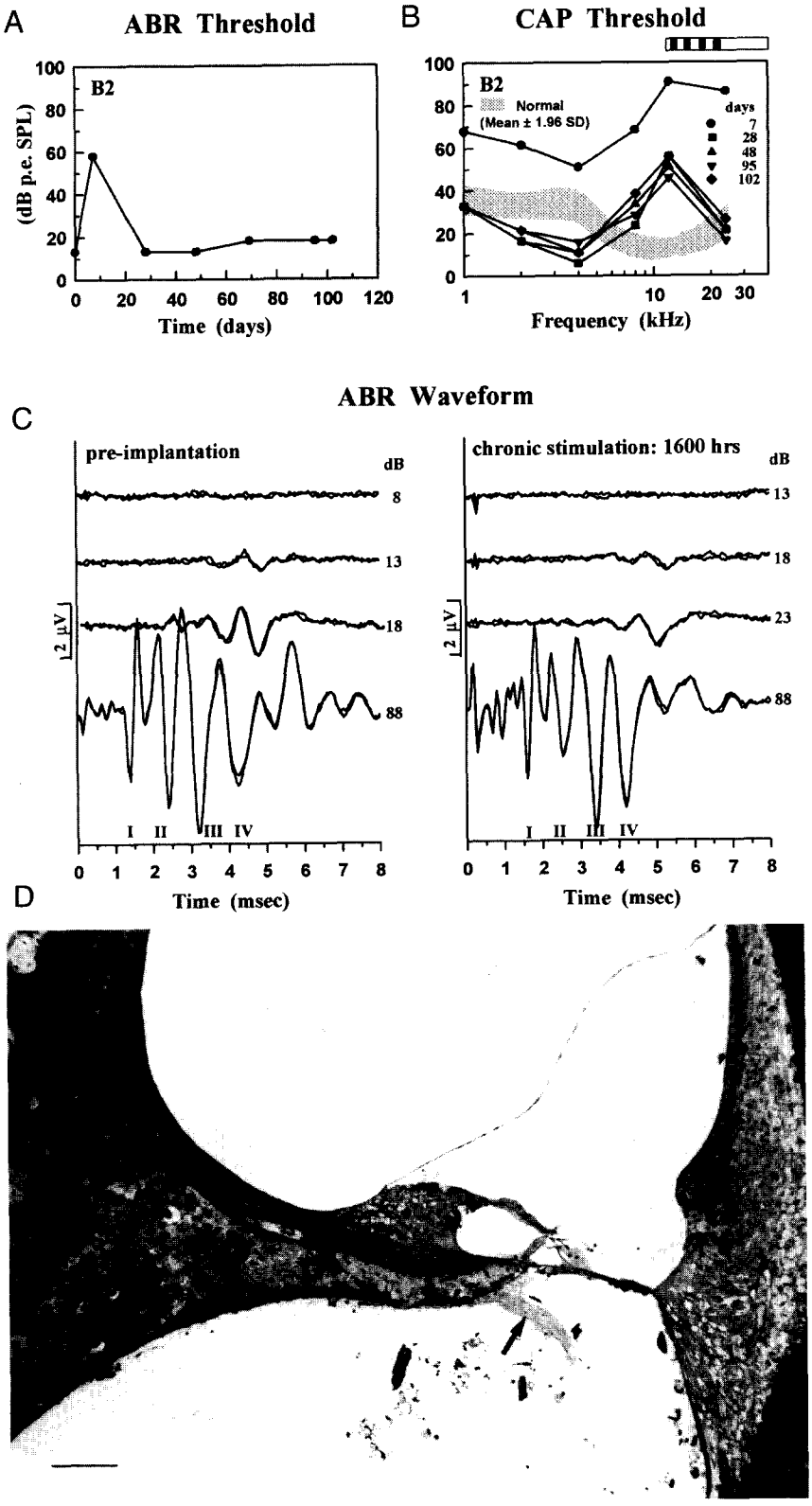
The care and use of animal's reported in this study were approved by The Royal Victorian Eye and Ear Hospital's Animal Research Ethics Committee ('Safety Considerations for Cochlear Prostheses', Project #92.001).

3. Results

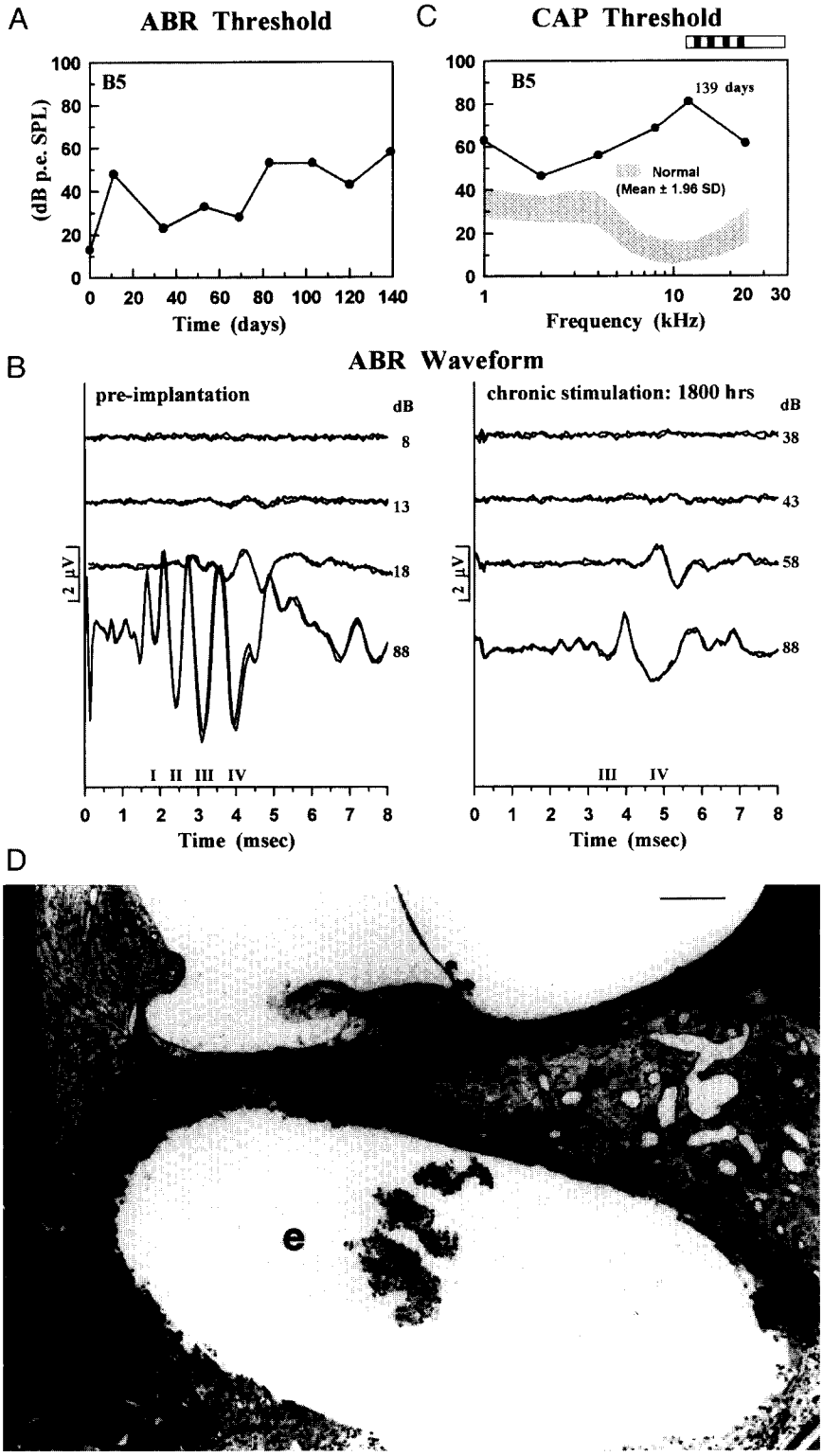
3.1. Cochlear physiology

3.1.1. Auditory brainstem responses

All cochleas exhibited an increase in click-evoked ABR thresholds over the first 2 weeks following implant surgery. Thereafter, the hearing status of each



ear could be classified into one of four groups. (1) ABR thresholds returned to within 15 dB of the pre-surgical threshold and were maintained at that level for the duration of the study. This was observed in 12 of the 26 ears (seven stimulated and five controls; Fig. 3A and Table 3). (2) ABR thresholds returned to within 15 dB of the pre-surgical level, however, underwent a subsequent elevation in threshold to a moderate hearing loss



(15–50 dB increase relative to the pre-surgical threshold). This was observed in two ears (one stimulated and one control; Fig. 4A). (3) ABR thresholds partially recovered to exhibit a moderate hearing loss (15–50 dB) for the duration of the study. A long-term moderate hearing loss was observed in six ears (two stimulated and four controls). (4) ABR thresholds exhibited a severe hearing loss (> 50 dB increase relative to the pre-surgical level) with no evidence of recovery following surgery. Three animals exhibited a severe bilateral hearing loss in this study (three stimulated and three control ears; Fig. 5A). Elevation in response threshold

Fig. 4. A: Click evoked ABR thresholds as a function of implantation time for cochlea B5. This cochlea was chronically stimulated for a period of 2100 h. The initial threshold (0 days) was recorded just prior to implantation. ABR threshold increased following implant surgery and recovered to near pre-surgical levels by 1 month; however, they subsequently increased over the experimental period. At completion of stimulation, a 45 dB elevation of threshold was observed. This delayed elevation in ABR thresholds may indicate an extensive inflammatory reaction within the scala tympani due to chronic infection rather than surgical trauma. B: Acoustically evoked CAP thresholds as a function of stimulus frequency monitored following an implant period of 139 days. The most extensive hearing loss was observed at 12 kHz, while moderate elevation of thresholds was observed at lower frequencies. C: Click-evoked ABRs from B5 recorded pre-implantation (left) and following 120 days of implantation and 1800 h of stimulation (right). The responses evoked from this cochlea exhibited a significant elevation in response threshold and an increase in latency of ≈ 1 ms in all waves compared with the responses recorded before implantation (left). The intensity of the click (in dB p.e. SPL) is shown to the right of each response. Two responses were recorded at each intensity. D: Photomicrograph of the upper basal turn of stimulated cochlea B5 (H and E) illustrated evidence of a grade IV inflammatory response presumably associated with low-grade infection. This inflammatory response resulted in a significant loss of hair cells and a moderate loss of spiral ganglion cells and peripheral processes in the basal turn of the cochlea. e, Electrode track. Bar = 100 μm.
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was associated with a decrement in response amplitude and an increase in the latency of all waves (Fig. 4C). Finally, the incidence of an elevation in the click-evoked ABR threshold was no greater for the chronically stimulated cochleas when compared with their unstimulated controls (Table 3).

3.1.2. *Compound action potentials*

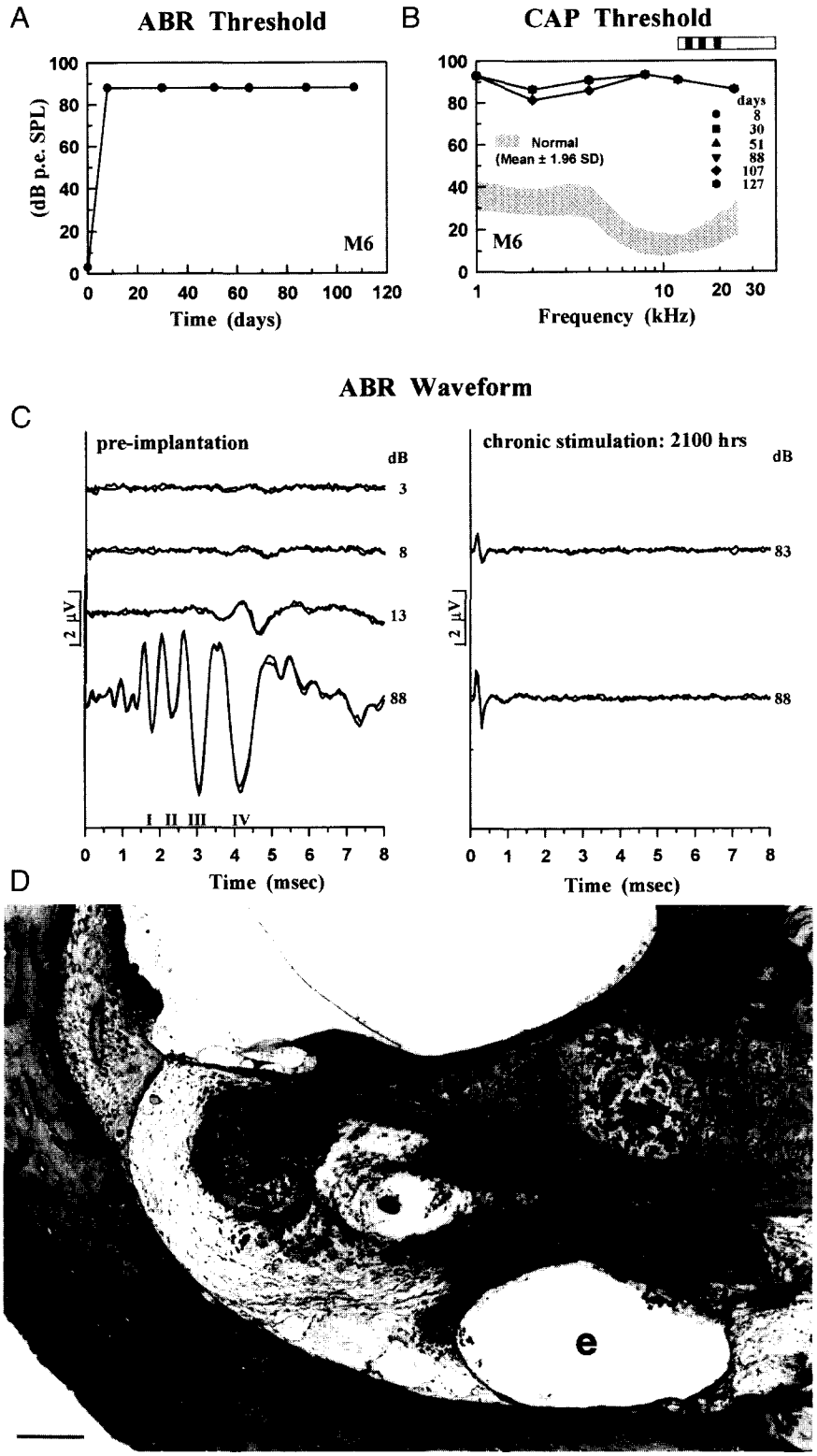
Frequency-specific CAPs, recorded from all stimulated cochleas, indicated that the most extensive hearing loss generally occurred in the high-frequency region of the cochlea. The majority of animals exhibited a moderate to severe loss in the 12–24 kHz region (i.e., adjacent to the electrode array). In lower-frequency regions (2, 4 and 8 kHz), thresholds appeared at normal or near-normal levels (Rajan et al., 1991) despite long-term cochlear implantation and electrical stimulation (e.g., Fig. 3B). Cochleas with a moderate or severe hearing loss as indicated by an elevation in the click-evoked ABR, exhibited a concomitant increase in CAP thresholds (Figs. 4B and 5B).

3.1.3. *Electrically-evoked auditory brainstem responses*

Typical examples of EABR waveforms for both monopolar and bipolar electrodes are shown in Fig. 6. The majority of EABRs evoked using monopolar electrodes and 25 μs/phase biphasic current pulses were restricted to maximum stimulus currents of less than 1.0 mA due to the ease in evoking large muscle activity at higher current levels. Using bipolar electrodes, responses to 50 μs/phase biphasic current pulses were presented at stimulus currents up to 2.0 mA. Stimulus-induced muscle activity was only observed in one animal using bipolar stimulation. Longitudinal EABR monitoring illustrated the general stability of both EABR threshold and the suprathreshold response during the chronic stimulation period. These results not only indicated the physiological viability of the auditory nerve but also allowed us to confirm that our chronic stimulus levels were above threshold for direct activation of the auditory nerve. EABR I/O functions (amplitude of wave IV versus stimulus current) were plotted for each stimulated electrode pair. Representative examples are illustrated in Fig. 7. The effects of stimulus duration (ranging from 0 to 2100 h) on EABR threshold was examined statistically using linear regression analysis. The results showed that for the majority of chronically stimulated electrodes examined (62%) there was no relationship between EABR threshold and stimulus duration ($P > 0.05$; Table 4). In addition, the effects of the duration of electrical stimulation on the gradient of the EABR I/O function were also evaluated statistically us-

Table 3
Click-evoked ABR thresholds (dB p.e. SPL)

Stimulated cochlea	Pre-surgery	Completion of stimulation	Control cochlea	Pre-surgery	Completion of stimulation
M1	8	88	MC1	8	53
M2	13	28	MC2	18	33
M3	−1	3	MC3	3	33
M4	13	43	MC4	13	58
M5	8	48	MC5	8	73
M6	3	88	MC6	−1	88
MB1	13	68	MBC1	8	88
B1	13	18	BC1	18	33
B2	13	18	BC2	13	33
B3	8	13	BC3	13	13
B4	13	18	BC4	8	53
B5	13	58	BC5	13	13
BM1	8	23	BMC1	8	23



ing linear regression analysis (Table 5). The results showed that for the majority of the electrodes examined (70%), there was no statistically significant relationship between gradient of the EABR I/O function and duration of electrical stimulation ($P > 0.05$).

3.2. Cochlear histopathology

3.2.1. Electrode insertion trauma

There was no evidence of trauma to the OSL, Reissner's membrane, stria vascularis or spiral ligament in

Fig. 5. A: Click evoked ABR thresholds as a function of implantation time for cochlea M6. This cochlea was chronically stimulated for a period of 2100 h. A permanent elevation in the ABR thresholds following implant surgery was observed in this cochlea. B: Acoustically evoked CAP thresholds as a function of stimulus frequency monitored longitudinally over the implant period of 127 days illustrates a severe, profound hearing loss at all frequencies tested. C: Click-evoked ABRs from M6 recorded pre-implantation (left) and following completion of the chronic stimulation program (right) at which stage the animal exhibited a profound hearing loss. The intensity of the click (in dB p.e. SPL) is shown to the right of each response. Two responses were recorded at each intensity. D: Photomicrograph of the upper basal turn of the stimulated cochlea M6 (H and E) illustrating evidence of a grade IV inflammatory response associated with a widespread loss of hair cells and a significant reduction of spiral ganglion cells and peripheral processes. e, Electrode track. Bar = 100 μm.
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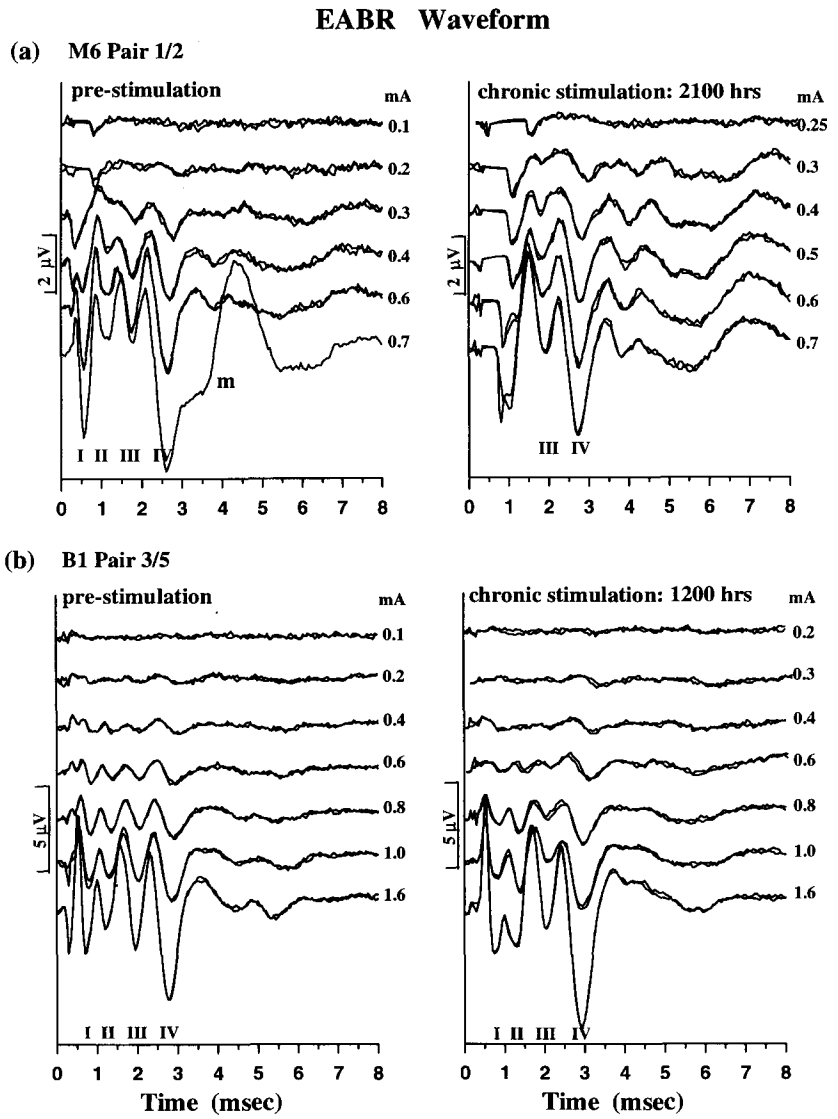

Fig. 6. a: Representative EABRs recorded from M6 before stimulation (left) and following 2100 h of electrical stimulation (right) using a monopolar stimulating electrode. The EABRs were evoked using 25 μs biphasic current pulses. The stimulus intensity (in mA) is indicated at the right of each set of responses (two responses are superimposed). WN masking of electrophonic activity was not used in this animal due to the permanent profound hearing loss following implantation (Fig. 5). Muscle activity (m) associated with facial nerve stimulation was observed at and above 0.7 mA in the pre-stimulation series. This muscle activity restricted the range of stimulus currents that could be used in monopolar electrode. The responses recorded at stimulus levels below 0.7 mA appeared similar to EABRs evoked from bipolar stimulating electrodes. The trough of the four waves of the EABR are indicated using Roman numerals. There was little change in threshold and morphology of the response following long-term electrical stimulation in this cochlea. b: Representative EABRs recorded from B1 before stimulation (left) and following 1200 h of electrical stimulation (right) using a bipolar scala tympani stimulating electrode. The EABRs were evoked using 50 μs biphasic current pulses. As this animal exhibited good preservation of hearing, continuous white noise was applied during EABR recordings at a level just sufficient to mask any electrophonic activity. Again, there were little change in threshold and morphology of the response following long-term electrical stimulation in this cochlea.

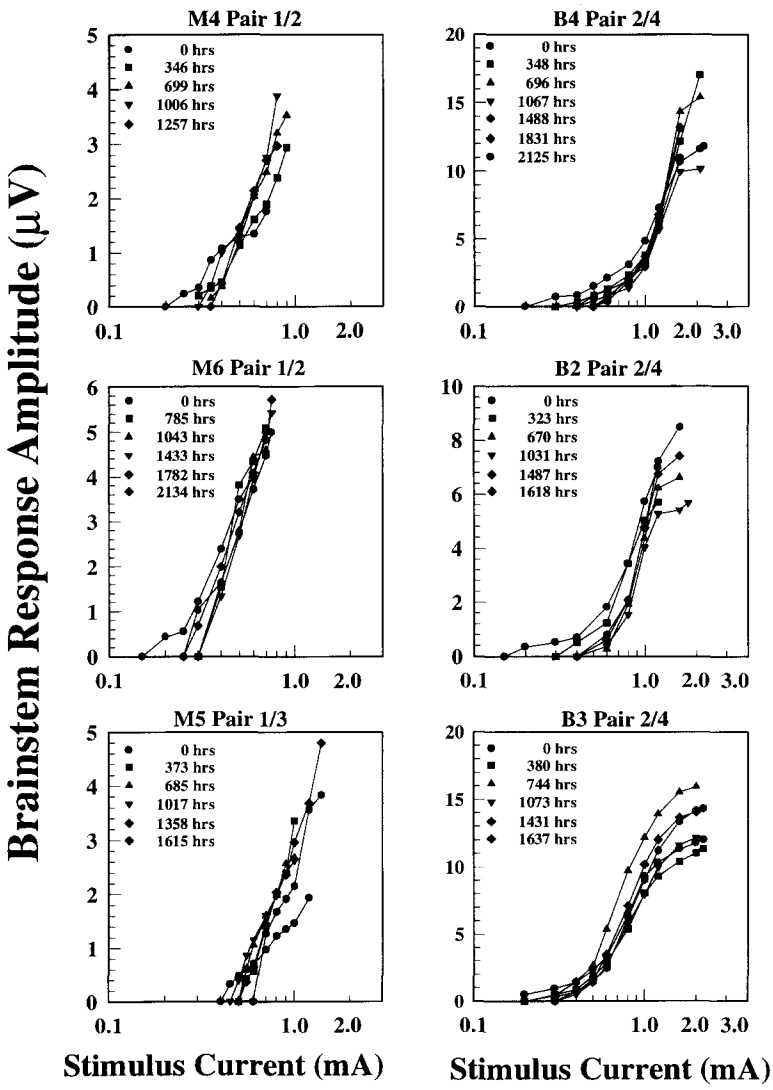

Fig. 7. Representative EABR I/O functions, plotting the peak-to-trough amplitude of wave IV of the EABR as a function of stimulus current for one electrode pair in monopolar stimulated cochleas (M4, M6, M5; left) and bipolar stimulated cochleas (B4, B2, B3; right). EABRs were recorded approximately every 300 h during the chronic stimulation program as indicated in the figure. The majority of cochleas exhibited a slight increase in threshold over stimulation time; however, EABR I/O functions remained stable throughout the long-term stimulation programme. Note the clear difference in morphology of the input-output function for monopolar compared with bipolar electrodes.

Table 4
Linear regression analysis of EABR thresholds vs. duration of electrical stimulation

Cochlea	Electrodes	R^2	P	Cochlea	Electrodes	R^2	P
M4	1/2	0.928	0.0054	B1	2/4	0.782	0.0295
	1/3	0.292	0.2021		3/5	0.316	0.2623
	1/4	0.284	0.2060	B2	2/4	0.248	0.1792
M5	1/2	0.442	0.1337		3/5	0.804	0.0097
	1/3	0.068	0.3385	B3	2/4	0.584	0.0823
	1/4	0.870	0.0443		3/5	0.858	0.0051
M6	1/2	0.219	0.1621	B4	2/4	0.740	0.0081
	1/3	0.283	0.1258		3/5	0.826	0.0029
	1/4	−0.032	0.4088	B5	2/3	0.857	0.0051
					4/5	−0.044	0.4287
				BM1	2/4	0.186	0.2603
					3/5	0.768	0.0805

M1, M2, M3, MB1 were not included in this table due to insufficient data.

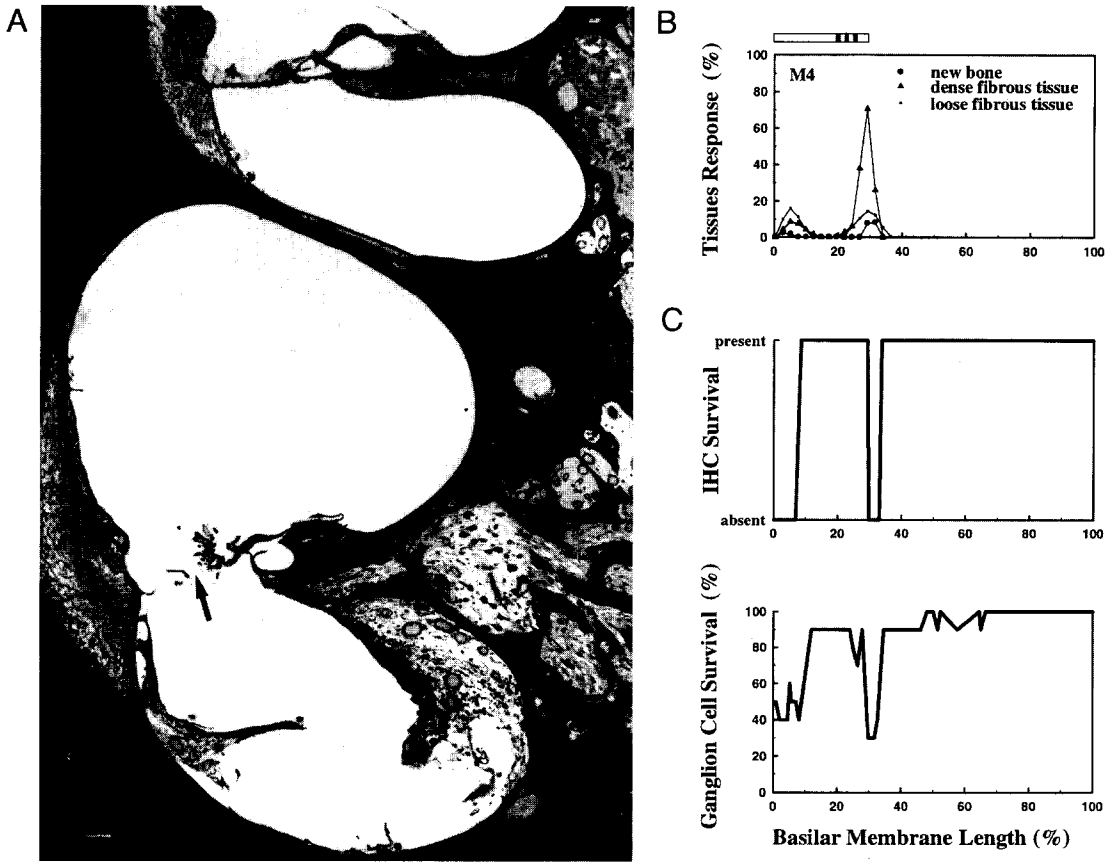

Fig. 8. A: Photomicrograph of the upper basal and upper middle turn of cochlea M4 (H and E) illustrating a localized rupture of the basilar membrane (arrow), associate with a mild fibrous tissue response and reduction of spiral ganglion cells and peripheral processes. This damage was located adjacent to the tip of the electrode array (31–32.5% of the basilar membrane length relative to the round window), and was one of only two cochleas in this study that exhibited electrode insertion trauma. Bar = 100 μm. B: Cochleogram of M4 illustrating the location and cross-sectional area of the tissue response within the scala tympani as a function of distance along the basilar membrane. Note that the most extensive new bone and dense fibrous tissue response was localized to the electrode tip, although some fibrous tissue and new bone was also observed at the electrode entry point close to the round window. The cross-sectional area of this tissue response is expressed as a percentage of the cross-sectional area of the scala tympani. C: Cochleograms illustrating the extent of hair cell and spiral ganglion cell loss throughout the cochlea. The most extensive region of loss occurred in the upper basal turn and was localized to the site of insertion trauma. As with the tissue growth, sensorineural elements were also reduced in the extreme cochlear base. Note, however, near normal hair cell and ganglion cell populations adjacent to the majority of the electrode array and apical to the site of trauma. This cochlea had been stimulated for 1258 h.

Table 5
Multiple linear regression analysis of EABR I/O functions

Cochlea	Electrodes	R^2	P	Cochlea	Electrodes	R^2	P
M2	1/2	0.933	0.5562	B1	2/4	0.828	0.1294
	1/3	0.934	0.7220		3/5	0.867	0.2968
	1/4	0.840	0.0180[a]	B2	2/4	0.718	0.4658
M3	1/4	0.728	0.5801		3/5	0.786	<0.0001
M4	1/2	0.898	0.1420	B3	2/4	0.654	0.2175
	1/3	0.902	0.0231		3/5	0.812	0.2243
	1/4	0.854	0.1056	B4	2/4	0.816	0.8592
M5	1/2	0.857	0.0004		3/5	0.907	0.5753
	1/3	0.841	0.0180	B5	2/3	0.315	0.7027
	1/4	0.815	0.1939		4/5	0.363	0.0119
M6	1/2	0.950	0.0034	BM1	2/4	0.786	0.7735
	1/3	0.949	0.0003		3/5	0.937	0.2877
	1/4	0.915	0.9352	MB1	2/4	0.914	0.5562
					3/5	0.901	0.0639

Dependent variable: gradient of EABR I/O function.
Independent variable: duration of electrical stimulation.
M1 and M3 (electrode 1/2, 1/3) is not included in this table due to insufficient data.

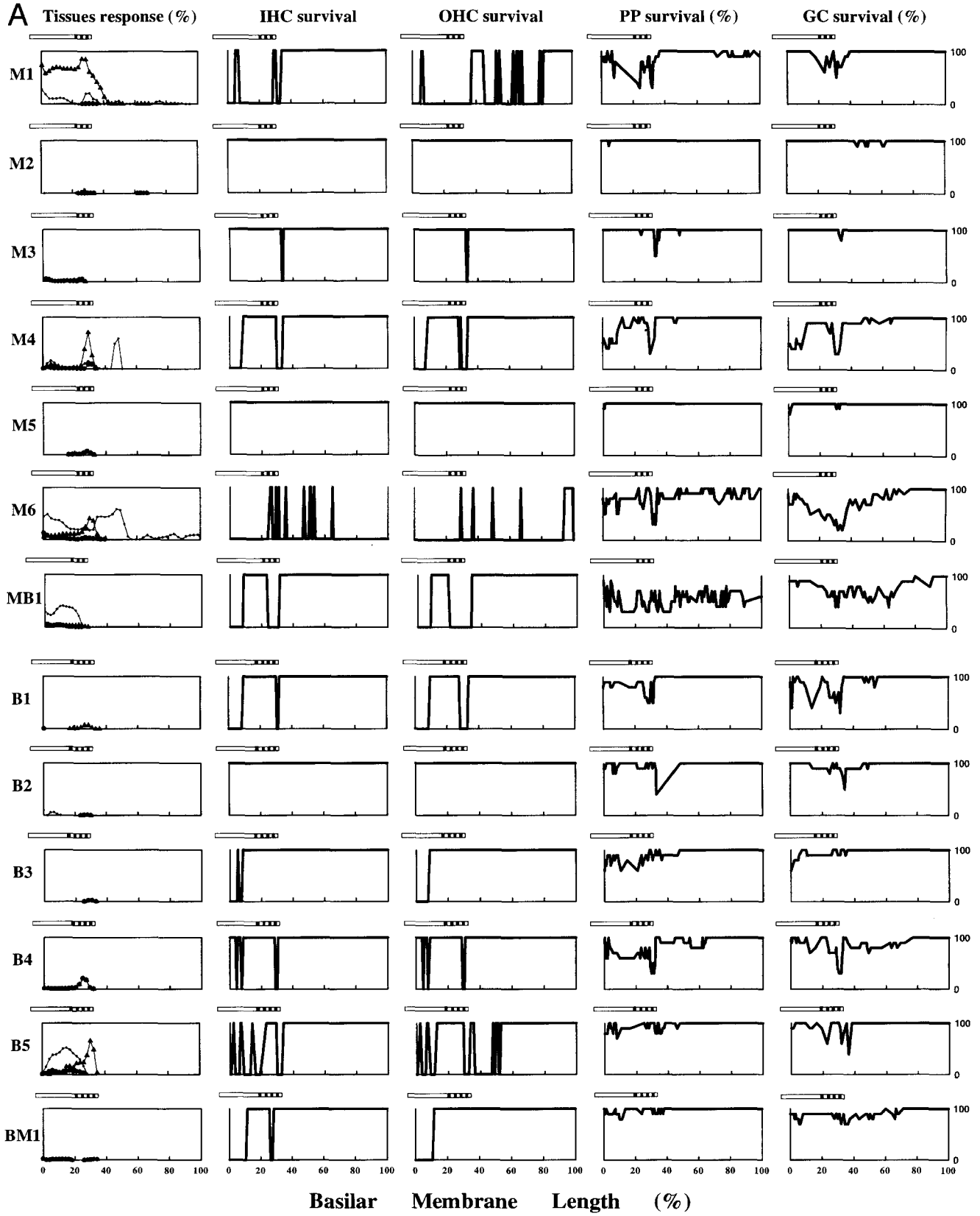

Fig. 9. Cochleograms of the 13 stimulated (left page) and 13 control cochleas (right page) in this study, illustrating the cross-sectional area of tissue response as a function of distance along the basilar membrane (symbol legend, see Fig. 8B); the extent of IHC and OHC survival, and the degree of peripheral process (PP) and spiral ganglion cell (GC) survival as a function of basilar membrane distance.

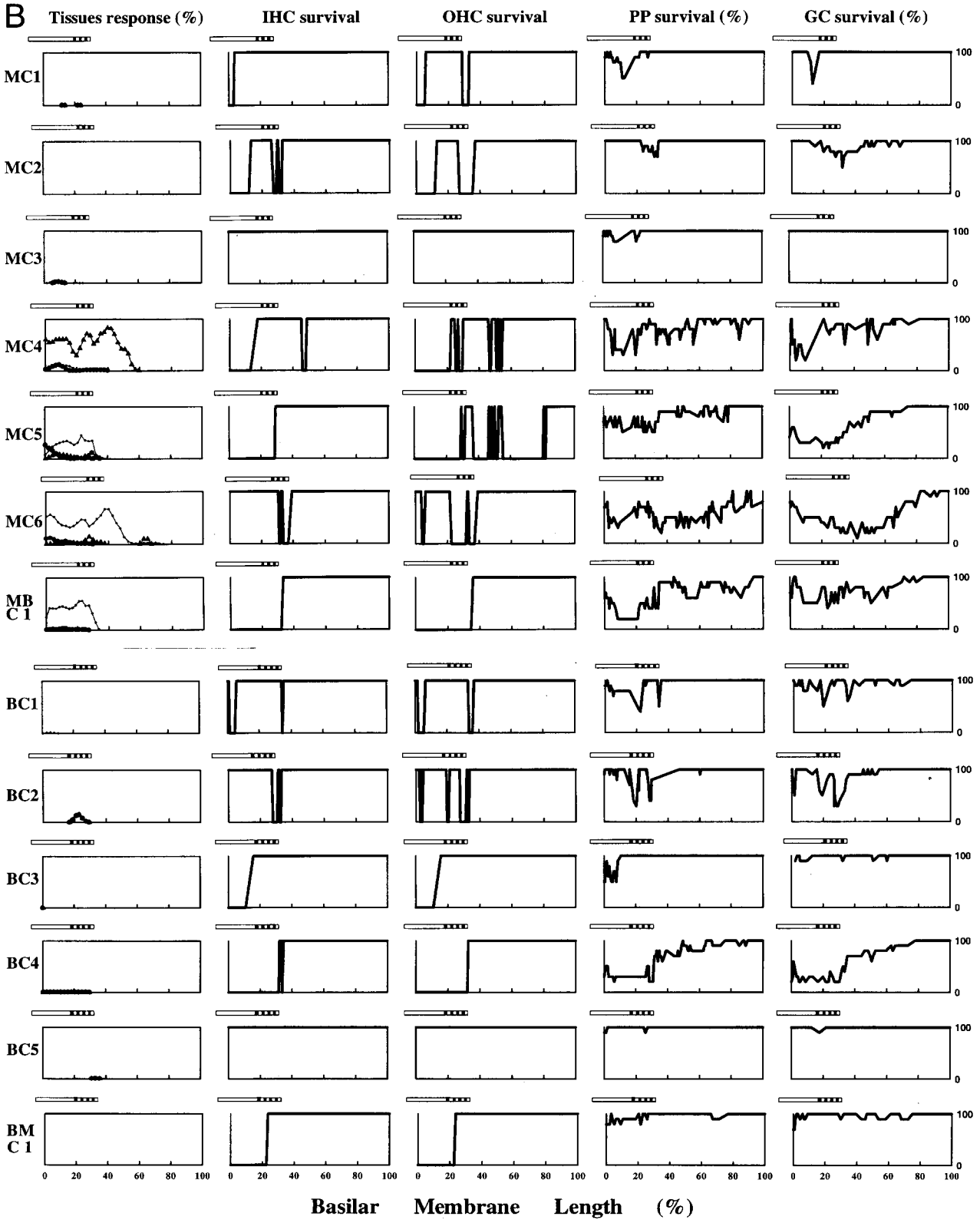

Fig. 9 (*continued*).

The approximate location of the scala tympani electrode array is also illustrated. Hair cell, peripheral processes and spiral ganglion cell survival are expressed as a percentage estimate relative to a normal population (100%).

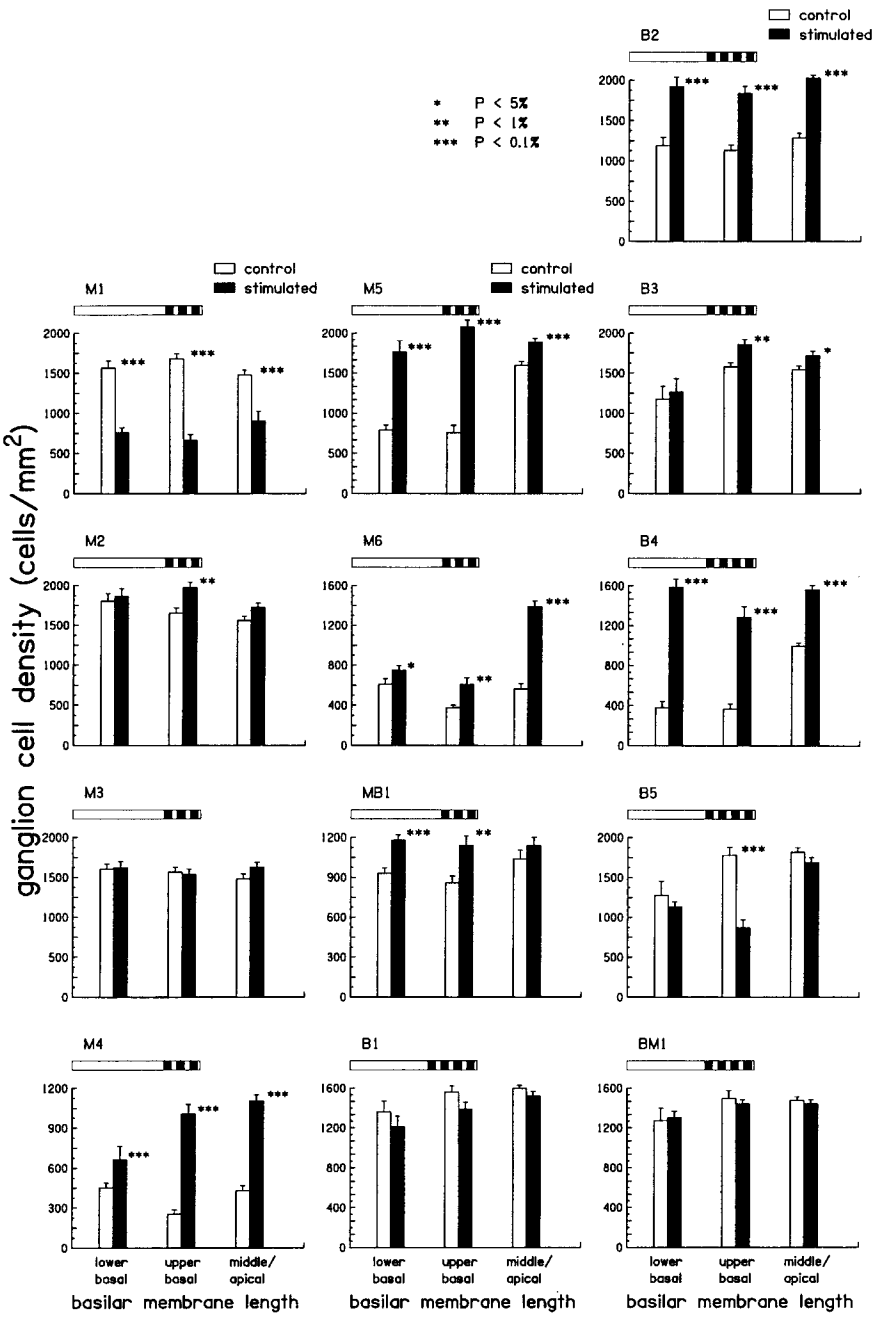

Fig. 10. Comparison of mean spiral ganglion cell densities for all 13 cats in this study. The results illustrate the ganglion cell density for three localized regions of the cochlea: (1) lower basal turn; (2) upper basal turn; (3) middle/apical turn. Note that the upper basal region represents the ganglion cell population adjacent to the stimulating electrodes as indicated by the presence of 4 bipolar or 3 monopolar electrodes. The control, unstimulated cochleas are illustrated as white bars while the stimulated cochleas are shown as black bars. While there was no overall statistically significant difference between stimulated and control pairs, in cases where there were significant differences, there was no trend to either an increased or decreased ganglion cell density associated with long-term electrical stimulation. Note the variations in the scale of the ordinate. Bar = 1 SE. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

any of the 26 implanted cochleas examined in the present study. However, a localized rupture (0.3–0.6 mm) of the basilar membrane was observed in two cochleas (M4, Fig. 8A; BC2). Graphic reconstruction of the cochleas confirmed that the trauma was located in the upper basal turn adjacent to the tip of the electrode (~31–32.5% of the basilar membrane length relative to the round window in M4; and 28–31% of the basilar membrane length in BC2). This trauma was associated with a localized soft tissue response and new bone growth in M4 (Fig. 8), while cochlea BC2 exhibited a minimal tissue reaction. A significant loss of hair

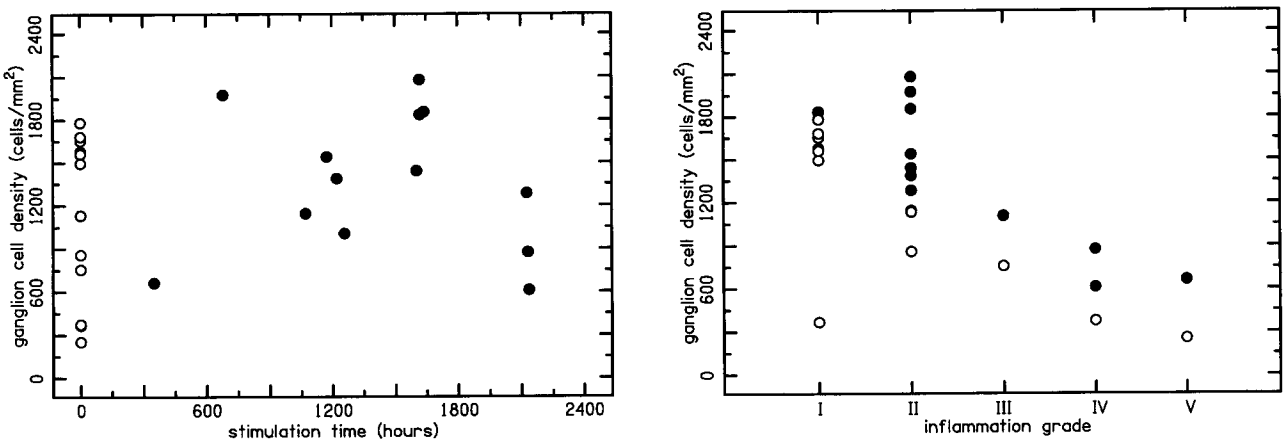

Fig. 11. Left: Mean ganglion cell density in the upper basal turn as a function of duration of electrical stimulation. Right: Mean ganglion cell density as a function of the degree of cochlear inflammation in upper basal turn. ●, Stimulated cochlea; ○, control cochlea.

cells, peripheral processes and spiral ganglion cells were observed in the region localized to the site of insertion trauma (Fig. 8).

3.2.2. *Inflammation*

Cochleograms illustrating the extent of tissue response as a function of distance along the basilar membrane were constructed for each of the 26 cochleas in this study (Fig. 9). Four stimulated cochleas and three control cochleas showed evidence of a moderate to severe inflammatory reaction (grade III–V; Table 2; Figs. 4 and 5D) in the basal turn based on increased numbers of acute or chronic inflammatory cells, or the presence of dense fibrous tissue and/or new bone. In addition, two of these cochleas (M1 and MC4) exhibited a more extensive inflammatory reaction that extended into the middle turn of the scala tympani. In these two cochleas, inflammatory cells had spread into the OSL, Rosenthal's canal, spiral ligament and organ of Corti. In addition to this inflammatory reaction, cochlea M4 exhibited a localized tissue reaction and new bone formation associated with insertion trauma (Fig. 8).

Ten control and nine stimulated cochleas showed minimal (e.g., loose fibrous tissue), or no evidence of inflammation in response to cochlear implantation (grade I–II; Table 2 and Fig. 9). In a number of these cochleas, the fibrous tissue capsule associated with the electrode array was difficult to detect. Tissue response was limited to a loose fibrous tissue reaction restricted to the region of the electrode array, or to a thin tissue capsule around the array (Fig. 3D).

A positive microbiological swab of the bulla was only observed in four of the 26 ears examined. The swab taken from the bulla of M2 cultured *Xanthomonas*, while swabs taken from the bullae of B2, BC1 and BC3 cultured a mycoplasma species. There was no correlation between the degree of inflammation observed in the cochlea and the microbiological results from the bulla swab. For example, none of the five cochleas that exhibited a severe inflammatory reaction (grade IV–V) in the scala tympani, had a positive microbiological culture of the bulla. In addition, while the microbiological results showed a positive culture in four bullae, histological results from these cochleas showed minimal or no evidence of inflammation (grade I–II). It is possible, therefore, that these positive cultures were not of pathological significance as *Xanthomonas* is widespread in the environment and many species of mycoplasmas are nonpathogenic organisms.

Statistically, the inflammatory reaction was slightly more severe in stimulated cochleas compared with control cochleas ($P = 0.0482$; Kruskal-Wallis ANOVA; Table 2); however, the severity of inflammation was not associated with implant duration ($P = 0.8268$; Kruskal-Wallis ANOVA). Statistical analysis also indicated that the degree of inflammation was not related to the extent of loss of IHCs and OHCs adjacent to the electrode array ($P = 0.2773$ and $P = 0.0926$, respectively; Kruskal-Wallis ANOVA). It would appear that the relatively high incidence of hair cell loss observed adjacent to the electrode array masks any association between inflammation and hair cell survival in this region of the cochlea. However, the relationship between the degree of inflammation and the extent of loss of OHCs was highly significant in the region apical to the electrode array ($P = 0.003$; Kruskal-Wallis ANOVA). Finally, comparison between the degree of cochlear inflammation and spiral ganglion cell density showed a significant relationship ($P = 0.0163$; Kruskal-Wallis ANOVA).

3.2.3. *New bone*

A small amount of new bone was observed in the scala tympani of the basal turn in 10 of the 26 cochleas examined. The new bone occupied less than 6.4% of the total volume of the scala tympani and was associated with either an inflammatory response (Fig. 9), or insertion trauma (Fig. 8). Two control cochleas MC4 and

MC5 showed new bone growth that had extended into the organ of Corti at limited sites. There was no evidence of new bone growth in either the scala media or scala vestibuli of any stimulated cochlea in the present study. Finally, the extent of the new bone formation appeared no greater in stimulated cochleas when compared with their unstimulated controls.

3.2.4. Inner and outer hair cell survival

Cochleograms illustrating the extent of IHC and OHC survival as a function of distance along the basilar membrane were constructed for each of the 26 cochleas (Fig. 9). Six of the cochleas examined in this study showed minimal or no evidence of IHC and OHC loss (M2, M3, M5, B2, MC3, BC5). It should be noted that four of these six cochleas had been chronically stimulated for periods of up to 1619 h. In 12 cochleas (six stimulated and six controls), hair cell loss was observed in the lower basal turn ($< 10\%$ of cochlear length), and/or in the region of the electrode tip ($\approx 30\%$ from round window). More extensive hair cell loss was observed in the basal turn of three control cochleas (MBC1, BC4, BMC1). While all these cochleas showed normal hair cell populations apical to the electrode array, five cochleas exhibited evidence of more extensive hair cell loss. Two stimulated (M1, B5) and two control cochleas (MC4, MC5) exhibited an OHC loss that had extended into the middle turn, while stimulated cochlea M6 exhibited a hair cell loss extending into the apical turn (Fig. 9). It should be noted, however, that no cochlea studied showed a complete loss of hair cells.

Statistical analysis showed, as expected, a highly significant difference in hair cell population between the region adjacent to the electrode array and the region apical to the array ($P < 0.0001$; Kruskal-Wallis ANOVA). Importantly, the extent of IHC and OHC loss, and the structure of the organ of Corti adjacent to the electrode array was not related to electrical stimulation ($P = 0.8581$, $P = 0.3025$ and $P = 0.6283$ respectively; Kruskal-Wallis ANOVA).

3.2.5. Peripheral process & spiral ganglion cell survival

Survival of peripheral processes and spiral ganglion cells, expressed as a percentage estimate of normal and plotted as a function of distance along the basilar membrane, is illustrated in Fig. 9. Although the peripheral processes exhibited more extensive loss than the spiral ganglion, the pattern of loss appeared very similar. Three of the 13 stimulated cochleas (M2, M3, M5), and four of the 13 control cochleas (MC3, BC3, BC5, BMC1) showed a normal ganglion cell population throughout all cochlear turns. Cochleas BM1, B2, B3 showed minor ganglion cell loss in the basal turn. Two cochleas (M4, BC2) exhibited localized moderate ganglion cell losses associated with electrode insertion trauma, together with a concomitant loss of peripheral processes and hair cells (Figs. 8 and 9). More widespread loss of peripheral fibres and spiral ganglion cells were observed in the basal turn of cochleas MB1, MBC1, M6, MC6, MC5. This loss was associated with a chronic inflammatory reaction (Table 2). The remaining cochleas exhibited ganglion cell losses that were generally confined to the localized regions of the lower basal turn or the electrode tip in the upper basal turn (Fig. 9). Apical to the electrode array the ganglion cell population appeared normal.

3.2.6. Spiral ganglion cell density

Fig. 10 illustrates the spiral ganglion cell density adjacent to and in regions both apical and basal to the scala tympani electrodes for each cochlea in this study. Statistical analysis of the spiral ganglion cell densities adjacent to the stimulating electrodes showed no significant difference in ganglion cell densities for cochleas stimulated using a monopolar versus a bipolar electrode configuration (Mann-Whitney U-test, $P = 0.88$; t-test, $P = 0.67$).

While there were often significant differences between the stimulated and control ganglion cell densities in some cochlear regions (Fig. 10), there was no overall trend of either a lower or higher ganglion cell density as a function of electrical stimulation ($P = 0.459$; Kruskal-Wallis ANOVA; Fig. 11). However, comparison of ganglion cell density with inflammation grade was, as noted above, statistically related ($P = 0.0163$; Kruskal-Wallis ANOVA; Fig. 11).

3.3. Electrode impedance

Representative graphs showing access resistance (R_a) and electrode impedance (Z_e) as a function of implantation time for five specific electrodes are illustrated in Fig. 12. The monopolar electrodes always exhibited lower impedance compared with the bipolar electrodes due to the larger surface area of the remote Pt disc electrode. Seven of 13 stimulated cochleas (M2, MB1, B1, B2, B3, B4, BM1) showed relatively low and stable R_a and Z_e for the duration of the implant period. In contrast, cochleas B5, M1 and M5 exhibited elevated R_a and Z_e values, which were generally associated with an extensive inflammatory reaction (Fig. 12). The remaining cochleas (M3, M4 and M6) showed slightly elevated R_a and Z_e values.

4. Discussion

Previous neurophysiological studies have demonstrated that continuous electrical stimulation of both the cochlea and the auditory brainstem at high stimulus rates and intensities could have adverse effects on the neuron's metabolism. This may lead to a reduction in

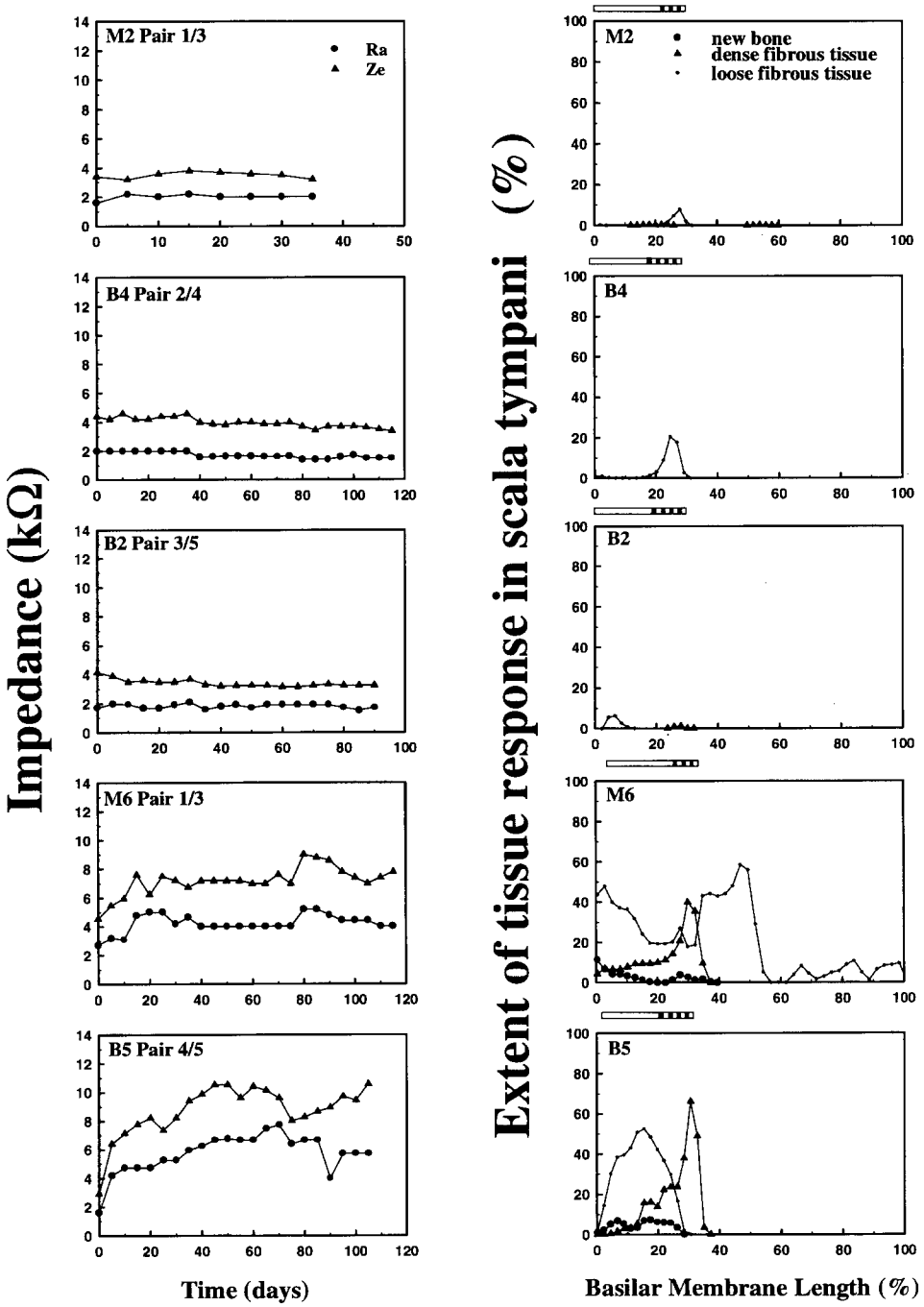

Fig. 12. Access resistance R_a and electrode impedance Z_e as a function of implantation time in five representative cochleas (left). The electrode impedance exhibited a good correlation with the density and extent of fibrous tissue and new bone formation around the electrode (right).

excitability and, if prolonged, result in irreversible neuronal damage (Miller et al., 1983; Shepherd and Clark, 1987; Shepherd et al., 1990a; McCreery et al., 1992; Killian et al., 1994; Tykocinski et al., 1995a). The extent of this decrement was found to be dependent on stimulus rate, duty-cycle and stimulus intensity (Tykocinski et al., 1995a).

In contrast with these previous acute studies, the present study has indicated that long-term intracochlear electrical stimulation, using carefully controlled charge-balanced biphasic current pulses presented at high rates and at intensities within the animal's behavioural dynamic range, does not adversely affect the adjacent organ of Corti, peripheral processes or spiral ganglion cell populations. In addition, longitudinally recorded EABRs remained relatively stable throughout the chronic stimulation period, showing no evidence of the significant stimulus-induced reductions reported in the previous acute investigations. Our present findings are supported by the recent results of Miller and his

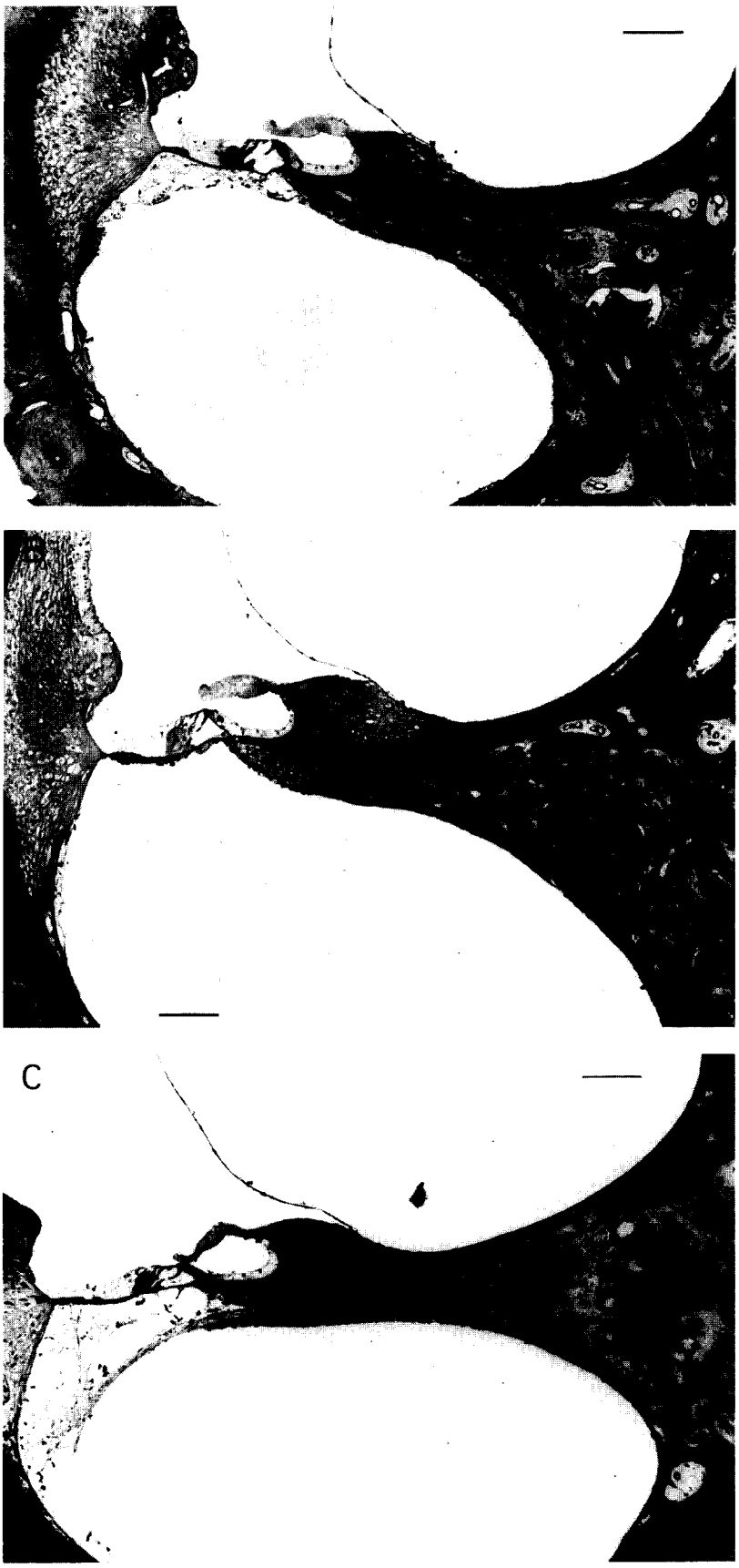
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Fig. 13. Photomicrographs of the upper basal turn of stimulated cochleas M2 (A), M3 (B), and BM1 (C). These cochleas had been implanted for periods of up to 108 days and stimulated for periods of up to 1600 h. Despite chronic electrical stimulation at rates of up to 2000 pps/channel, these cochleas exhibited a minimal tissue reaction, a minor loss of hair cells and peripheral processes, and no evidence of a significant ganglion cell loss. Thionin. Bar = 100 μm.
←

colleagues (Mitchell et al., 1997). They reported no evidence of stimulus-induced neural damage following 1000 h of electrical stimulation using charge-balanced biphasic current pulses at stimulus rates of up to 2750 pps and charge densities of 5 $\mu C \cdot cm^{-2}$ per phase.

While many aspects of the stimulus paradigms used in both the present study and by Mitchell et al. (1997) are similar to those used in the acute studies cited above, there also exist two major differences. First, the chronic studies used significantly lower stimulus intensities and somewhat lower duty cycles. It would follow that at these behaviourally accepted stimulus intensities, the entrainment of the auditory nerve to the electrical stimulus would be far less than that observed in the acute studies. Presumably, this reduction in stimulus driven neuronal activity has contributed to the lack of neural damage observed in the present study. Second, unlike the single electrode pair used in the acute studies, several sites of non-simultaneous stimulation were used in the present study. This more accurately models the situation in a multichannel cochlear implant, where electrically evoked neural activity is distributed over a wide region of the auditory nerve, reducing the extent of continuous neural activity in any one region.

There is now abundant evidence that an increase in neuronal activity to levels well beyond the physiological norm can result in an increase in stimulus-induced neural damage. It would appear that stimulus-induced neuronal hyperactivity can lead to metabolic stress resulting in a loss of homeostasis and ultimately cell death (Yarowsky and Ingvar, 1981; McCreery and Agnew, 1983; Sloviter, 1983; Shepherd and Clark, 1987; Scharfman and Schwartzkroin, 1989; Shepherd et al., 1990a; Agnew et al., 1993; Wasterlain et al., 1993; Tykocinski et al., 1995a). Even in the absence of electrically evoked neuronal activity, high rate electrical stimulation can cause prolonged depolarization of the neural membrane as a result of the non-linear properties of ion channels (Bromm and Frankenhaeuser, 1968). Prolonged periods of partial depolarization could also result in loss of ionic homeostasis and ultimately lead to cell death. For these reasons, careful evaluation of the safety of speech-processing strategies based on high stimulus rates is particularly important.

4.1. Electrical stimulation and cochlear pathology

The type and severity of the tissue response observed in the present study was similar to our previous observations (Shepherd et al., 1983, 1990b; Ni et al., 1992). Minimal tissue response within the scala tympani was observed in 19 of the 26 cochleas. If present at all, the tissue response in these cases was confined to a fine loose fibrous tissue or thin electrode-tissue capsule, and was usually associated with a minimal loss of sensory hair cells, peripheral processes or spiral ganglion cells (Fig. 13). This finding highlights the biocompatible nature of both the electrode array and the stimulus waveform. A more widespread tissue response, observed in seven cochleas in the present study was associated with a moderate–severe inflammatory reaction, including in some cases the presence of acute inflammatory cells. Presumably this type of reaction was associated with a low-grade infection.

Statistically, the stimulated cochleas examined in the present study exhibited a more extensive tissue response when compared with the control cochleas. However, it must be emphasized that this difference can be attributed to the fact that the majority of control cochleas exhibited a Grade I inflammatory response (no tissue reaction within the scala tympani) while the majority of stimulated cochleas exhibited a Grade II response (minimal fibrous tissue capsule associated with the electrode array). In terms of cochlear pathology, there was little difference between these two inflammation grades. It is also important to note that eight of the 13 stimulated cochleas exhibited minimal inflammation (Grade I–II) despite being electrically stimulated at high stimulus rates for periods of up to 2127 h (Table 1 and Figs. 9 and 13). We have previously reported an increased fibrous tissue reaction following chronic electrical stimulation in neonatally deafened cochleas (Shepherd et al., 1994a). This tissue response was more extensive than that observed in the present study, suggesting that the young deafened cochleae may be predisposed to a more vigerous fibrous tissue reaction in response to cochlear implantation and electrical stimulation. The mechanisms associated with an increase in fibrous tissue response remains to be elucidated.

As in our previous chronic stimulation studies (Shepherd et al., 1983, 1990a, 1994a; Ni et al., 1992) the spiral ganglion cell density was highly related to the grade of inflammation. In addition, the OHC loss apical to the electrode array was highly dependent on the degree of inflammation, reflecting the widespread nature of the moderate–severe inflammatory response observed in the present study. Finally, it is worth noting that of the seven cochleas exhibiting a moderate-to-severe inflammatory reaction (Grades III–V), four were

from two animals that showed an increased inflammatory reaction bilaterally (M4 and M6). This finding may reflect an individual difference in immune response.

New bone formation, observed in ten of the 26 cochleas examined in the present study, was limited to small regions ($<6.4\%$) within the basal turn scala tympani. While osteoneogenesis was associated with chronic inflammation and trauma, it was not associated with electrical stimulation per se, as the most severe new bone formation occurred in control cochlea MC5 (Fig. 9). Moreover, seven of the 13 stimulated cochleas examined histologically showed no evidence of new bone formation. This finding is consistent with our previous chronic stimulation studies using lower stimulus rates (Shepherd et al., 1983, 1994a; Ni et al., 1992; Clark et al., 1995).

Although OHCs exhibited more extensive histopathological changes than IHCs, the overall pattern of hair cell degeneration was similar (Fig. 9). In general, hair cell population appeared normal apical to the electrode array, while the most extensive hair cell loss was observed in the lower basal turn ($<10\%$ of cochlear length) and in the region of the electrode tip ($\approx 30\%$ of cochlear length). Statistically, there was a highly significant difference in the hair cell population adjacent to the electrode array compared with the region apical to the electrode. The hair cell loss observed in both the lower basal turn and in the region of the electrode tip, was presumably a result of mechanical damage during the surgical procedure and/or the physical presence of the array close to the basilar membrane. This has been observed previously using both free-fit electrode arrays (Shepherd et al., 1983, 1990a; Ni et al., 1992), as well as electrode arrays designed to occupy a larger proportion of the scala tympani (Leake et al., 1990).

It is unlikely that the effects of chronic electrical stimulation per se contribute to hair cell loss since five cochleas that had undergone chronic electrical stimulation exhibited no evidence of hair cells loss in the region adjacent to the stimulating electrodes (Fig. 13). Previous reports have described hair cell survival adjacent to scala tympani electrode arrays following long-term implantation (Simmons, 1967; Clark, 1977) and chronic electrical stimulation at low stimulus rates (Shepherd et al., 1983; Ni et al., 1992). The present study indicates that long-term electrical stimulation using high stimulus rates and stimulus levels within behavioural limits does not adversely affect hair cell survival.

From the present results, it does not appear that the chronic electrical stimulation at high stimulus rates result in the loss of peripheral processes or spiral ganglion cells. Six of the 13 stimulated cochleas showed no or minor ganglion cell loss in the basal turn despite having being stimulated for periods of up to 1640 h (Fig. 13). Although the statistical analysis showed that there were significant differences in the spiral ganglion cell density between individual stimulated and control cochleas for some regions (Fig. 10), there was no overall trend of either increased or decreased ganglion cell density associated with long-term stimulation, and therefore, there was no significant relationship between spiral ganglion cell density and duration of electrical stimulation ($P=0.459$; Kruskal-Wallis ANOVA; Fig. 11). This finding indicates that spiral ganglion cell survival was independent of long-term electrical stimulation using charge-balanced biphasic current pulses at rates of up to 2000 pps and charge densities of up to 15 $\mu C\ cm^{-2}$ geom. per phase.

The present findings are in agreement with a recent study by Mitchell et al. (1997), who evaluated the effects of high rate electrical stimulation in the deafened guinea pig. Using a monopolar Pt-Ir scala tympani electrode and continuous pulsatile electrical stimulation at a 60% duty cycle for 1000 h, they concluded that high-rate electrical stimulation, at charge densities of 5 $\mu C\ cm^{-2}$ geom. per phase and stimulus rates of up to 2750 Hz, does not result in damage to the auditory nerve or cochlea in general. Moreover, they reported that electrical stimulation provided an enhancement of spiral ganglion cell survival in the stimulated cochlea compared with the implanted, unstimulated control cochlea. This finding is in accordance with a number of previous studies following chronic stimulation at lower rates (Lousteau, 1987; Hartshorn et al., 1991; Leake et al., 1991), although a recent report from our laboratory has shown no evidence of enhanced ganglion cell survival with chronic electrical stimulation in neonatally deafened kittens (Shepherd et al., 1994a).

The pattern of loss of spiral ganglion cells observed in the present study was also reflected in the loss of peripheral processes. In cochleas MB1, MBC1, MC6, M6, MC5, a moderate to extensive reduction of peripheral processes and spiral ganglion cells was associated with a chronic inflammatory reaction. In two of 26 cochleas, a significant localized loss of hair cells, peripheral processes and spiral ganglion cells was associated with insertion trauma, a finding consistent with previous studies in both animal and human (Simmons, 1967; Clark, 1977; Schindler et al., 1977; Johnsson et al., 1982; Leake-Jones and Rebscher, 1983; Shepherd et al., 1983, 1994a; Sutton and Miller, 1983). These studies found that traumatic electrode insertion would further aggravate the ongoing degeneration of neuronal elements, even in profoundly deaf cochleas, and highlights the importance of developing an electrode array and insertion protocol that minimizes the incidence of trauma to either the basilar membrane or osseous spiral lamina.

Six of the 26 cochleas examined showed regions where peripheral processes were lost but the adjacent IHC populations appeared near-normal (B2, B3, MC1, MC3, MC6, and MBC1). The mechanism underlying

this pathology remains unclear. While inherent difficulties associated with estimating populations of peripheral processes may explain examples in which there is a relatively small difference between peripheral process and IHC survival (e.g., MC3); other cochleas exhibited large differences (e.g., B2, MC6 and MBC1) which are clearly unrelated to variability in measurement estimates. It should be noted, however, that large differences between IHCs and peripheral process survival appeared to be independent of both electrical stimulation and the degree of inflammation.

4.2. Monopolar versus bipolar stimulation

EABRs evoked by monopolar electrodes exhibited a high gradient single-limbed I/O functions. In contrast, responses evoked by bipolar electrodes exhibit a two-limbed I/O function; a low gradient, near threshold limb, followed by a higher gradient limb at current levels some what above threshold (e.g., Fig. 6). Similar observations have been reported by others (Merzenich and White, 1977; Marsh et al., 1981; Black et al., 1983; Shepherd et al., 1983, 1994a; van den Honert and Stypulkowski, 1986; Abbas and Brown, 1991). This difference presumably reflects the greater current spread associated with monopolar stimulation compared with bipolar stimulation.

Consistent with this observation was the finding that muscle activity in response to facial nerve stimulation was observed at stimulus levels in the range 0.65–0.90 mA at 25 μs/phase for the majority animals using a monopolar electrode configuration (Fig. 6). The extent and threshold of this muscle activity was independent of the site of the extracochlear electrode (the extracochlear electrode was placed either inside, or in muscle just outside the ipsilateral bulla). The presence of muscle activity limited the range of the stimulus current that could be used in this group of animals. In the bipolar group, facial nerve stimulation was only recorded in one cat at current levels greater than 1.7 mA and 50 μs per phase. In general, bipolar scala tympani stimulation does not evoke non-auditory activity. While facial nerve activity evoked from experimental animals using a monopolar electrode configuration has been observed previously (van den Honert and Stypulkowski, 1986; Shepherd et al., 1994a), our clinical experience has indicated a much lower incidence of facial nerve stimulation in patients using monopolar electrodes. Presumably, the current levels required to evoke such activity are beyond the patient's maximum comfortable level for electrical stimulation of the auditory nerve, although these differences may also reflect significant anatomical and physiological differences between human and cat.

Statistical analysis showed that there was no significant difference in the spiral ganglion cell density for ganglion cells adjacent to monopolar stimulating electrodes when compared with similar populations adjacent to bipolar electrodes. This finding has important implications for the clinical application of high-rate speech-processing strategies. It indicates that these strategies can be safely implemented using monopolar or bipolar electrode arrays, despite the fact that bipolar electrodes operate at higher stimulus intensities and produce more localized neural excitation profiles.

4.3. Correlation between cochlear histopathology and electrophysiology

Not unexpectedly, we observed a good correlation between ABR thresholds and the degree of hair cell survival observed histologically. For example, half of the cochleas examined in the present study showed less than a 15 dB elevation in the click-evoked ABR threshold at completion of the stimulation program compared to their pre-implant click thresholds, even through more than 50% of these cochleas were electrically stimulated. Histopathologically, these cochleas showed a near-normal population of IHCs and OHCs in the upper basal turn adjacent and apical to the electrode, and a minimal tissue response adjacent to the electrode array. In contrast, six cochleas — half of which were chronically stimulated — exhibited an extensive loss of IHCs and OHCs adjacent to the electrode array and also showed a permanent elevation of more than 50 dB in the click-evoked ABR threshold. The changes in click-evoked ABR waveform included a significant decrement in the response amplitude, an elevation in response threshold, and a significant increase in the latency of all waves (e.g., Fig. 4C). While a decrement in response amplitude and increase in the response latency was also observed in animals whose click-evoked ABR thresholds were maintained at near-normal levels, the extent of these changes were less than that observed in cases exhibiting a more extensive hearing loss.

The longer response latencies suggest that the major component contributing to the response was located in a lower-frequency, more apical region of the cochlea (Don and Eggermont, 1978; Evans and Elberling, 1982; Shepherd and Clark, 1985). This was confirmed by recording the frequency-specific CAPs in all stimulated cochleas (e.g., Figs. 3B and 4B). These data showed that the most extensive hearing loss occurred in the high-frequency basal region even in those cochleas that exhibited normal thresholds to the click-evoked ABR (Fig. 3A). These changes in threshold appeared to be related to a loss of hair cells in the high-frequency basal region of the cochlea and/or a combination of the effects of fibrous tissue and the presence of the electrode array on the mechanical properties of the cochlea.

Maintenance of near-normal click-evoked ABR thresholds in the majority cochleas in our study suggests that hair cells — at least apical to an implanted electrode array — cannot only survive but apparently function at a near-normal sensitivity. This has also been observed in previous studies using low stimulus rates in normal-hearing cats (Shepherd et al., 1983; Ni et al., 1992). A number of studies have reported histological evidence of hair cell survival adjacent or — more typically — apical to a chronically implanted scala tympani electrode array in experimental animals (Simmons, 1967; Walsh and Leake-Jones, 1982; Shepherd et al., 1983, 1995; Leake et al., 1990; Ni et al., 1992). Moderate IHC and OHC survival has also been reported following examination of a temporal bone from a cochlear implant patient who had received his device 7 months prior to his death (Zappia et al., 1991). Moreover, surgical techniques have recently been developed to minimize the effects of cochlear implantation on residual hearing (Laszig and Lehnhardt, 1994). The long-term survival of hair cells apical to the electrode array may mean that electrophonic activation of residual hair cells could be used to provide cochlear implant patients with additional pitch and temporal cues useful for speech comprehension (McAnally et al., 1993).

The EABR data in the present study showed that chronically stimulated auditory nerve fibres remained physiologically viable over the period of stimulation. While the response amplitude generally remained stable during the long-term stimulation program (Table 5), approximately one-third of the stimulating electrodes examined exhibited a statistically significant increase in EABR threshold over the duration of the stimulation program (Table 4). It should be noted, however, that there was no correlation between ganglion cell survival and elevation in EABR threshold. Moreover, we observed no correlation between ganglion cell survival and a reduction in the gradient of the EABR I/O function. For example, cochlea M6 showed extensive loss of spiral ganglion cells; however, both EABR thresholds and suprathreshold responses remained stable for the duration of the chronic stimulation program. In general, cochleas that exhibited a significant increase in EABR threshold as a function of implantation time, did not exhibit a significant reduction in the gradient of the I/O function. Indeed, the only electrode pair to exhibit both a statistically significant increase in threshold and a reduction in response gradient had a normal ganglion cell density adjacent to the electrode array (cochlea B2 electrodes 3/5). In an attempt to correlate some parameter of the EABR with ganglion cell survival we performed the Pearson Product Moment Correlation comparing the upper basal turn spiral ganglion cell density with a variety of EABR parameters for all electrode combinations in which EABRs were evoked (EABR amplitude at 6 dB above threshold and at set current levels of 0.6, 1.0 and 1.6 mA; EABR threshold; change in EABR threshold over implantation time; and the gradient of the EABR growth response). No parameter tested exhibited a significant correlation with spiral ganglion cell density. This finding is consistent with previous studies that have unsuccessfully attempted to correlate wave IV of the EABR with ganglion cell survival (Shepherd et al., 1983; Stypulkowski et al., 1986; Hall, 1990), and raises the question of the potential clinical application of EABRs in determining VIIIth nerve population in cochlear implant patients.

It is conceivable that variations in the extent of the tissue response adjacent to the electrode array may have contributed to the variations observed in EABRs recorded longitudinally, as an increased tissue capsule surrounding the electrode array may alter the proportion of current shunting directly between stimulating electrodes. However, examination of the tissue response adjacent to each electrode array showed no evidence of such a relationship. Moreover, there was no correlation between the EABR threshold or suprathreshold response and electrode impedance (a measure of the tissue response adjacent to the electrodes). Finally, it has also been suggested that loss of hair cells may alter the resistive pathways within the cochlea, leading to changes in EABR characteristics (Black et al., 1983). Again, we observed no clear relationship between hair cell loss and statistically significant changes in EABR threshold or suprathreshold responses. On the basis of these findings it would seem prudent to record wave I of the EABR or the electrically evoked compound action potential, which has been shown, in the rat, to quantitatively correlate with spiral ganglion survival (Hall, 1990).

The general stability of the EABR I/O functions observed in the present study contrast with the significant increases in response gradient we have observed in previous studies using neonatal animal models (Ni et al., 1992; Shepherd et al., 1994a). We had previously postulated such changes were a result of modification in the intracochlear current distribution due to tissue encapsulation associated with the implanted electrode array. However, given that the range of tissue response in the present study are similar to that observed in the previous studies, it is possible that the changes observed in neonatal animals are associated with maturation.

4.4. Electrode impedance and cochlear histopathology

The access resistance (R_a) and electrode impedance (Z_e), measured at completion of the chronic stimulation program, exhibited a close correlation with the degree of tissue reaction observed around the electrode array. Cochleas MB1, M2, BM1, B4, B2, B3, B1, for example, showed relatively low and stable R_a and Z_e measurements (R_a 1–3 kΩ; Z_e 2.5–7 kΩ). The electrodes from these cochleas were associated with a minimal tissue

response. In contrast, cochleas B5 and M1 exhibited elevated R_a and Z_e values (R_a 2.0–7.0 kΩ; Z_e 6–12 kΩ). In both cases, the electrode array was generally encapsulated by a dense fibrous tissue capsule associated with chronic inflammatory cells and a small amount of new bone. Cochleas M4 and M6 showed a slightly increased R_a (2.5–5 kΩ) and Z_e (6–9 kΩ) values, and were associated with a moderate-dense fibrous tissue reaction around the electrode array. The most obvious association between electrode impedance and cochlear histology in the present study, was the density and extent of the fibrous tissue, inflammatory cells and new bone formation surrounding the electrode array. These results are consistent with our previous observations of electrode impedance (Shepherd et al., 1990a, 1994a; Shute, 1990; Ni et al., 1992; Clark et al., 1995). Moreover, they highlight the potential application of this technique in the clinical setting in order to predict the extent of the tissue response within the implanted cochlea.

4.5. *Direct current stimulation*

Previous studies have shown that DC stimulation could induce histological damage, alter auditory nerve activity, and change EABR morphology (Dodson et al., 1987; Cousillas et al., 1988; Shepherd et al., 1991). More recently, Tykocinski et al. (1995a,b) observed an increase in residual DC with increasing stimulus rate at intensities significantly above maximum clinical levels, despite using charge-balanced biphasic current pulses and shorting the electrodes between pulses. They also reported a stimulus-induced hearing loss in prior normal-hearing animals following acute intracochlear stimulation using DC levels of 7–12 μA. In the present study, using stimulus intensities operating within the animals behavioural dynamic range, DC levels of less than 0.1 μA were recorded, which is the same as we have previously observed in chronic stimulation studies using lower stimulus rates (Shepherd et al., 1990b, 1994a; Ni et al., 1992). While it is clear that these levels of DC do not result in cochlear damage, maximum safe levels of DC are yet to be clearly established.

5. Conclusion

The physiological and histopathological results in this study have indicated that chronic intracochlear electrical stimulation using either monopolar or bipolar stimulation at rates of up to 2000 pps/channel, using carefully controlled charge-balanced biphasic current pulses, including electrode shorting techniques to minimise DC, did not adversely affect the auditory nerve fibres, or the cochlea in general. This study provides an important basis for the safe application of improved speech processing strategies based on high rate electrical stimulation.

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