THE COCHLEAR PROSTHESIS:

SAFETY INVESTIGATIONS

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in fulfilment of the requirements
for the degree of

Doctor of Philosophy

in the

Department of Otolaryngology

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September 1986
To my wife and children
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The development of cochlear prostheses has heralded a significant change in the management of the profound and totally deaf. These devices electrically stimulate residual auditory nerve fibres so as to provide patients with important speech cues, therefore assisting their understanding of running speech.

This thesis forms part of a safety and efficacy investigation of the Nucleus Limited Cochlear prosthesis. A significant portion of this work was included in a successful submission by Nucleus Limited to the United States Food and Drug Administration for approval to market the device in the United States.

This thesis is less than 100,000 words. It is the result of original work done by the author except where referred to in the text. This work has not been carried out in collaboration with another person, and has not been submitted or accepted for an award of any other degree at any university. Some sections of this work have been published previously;


Shepherd, RK, Clark, GM, Black, RC & Patrick, JF (1983). The histopathological effects of


ABSTRACT OF THESIS

Title: The Cochlear Prosthesis: Safety Investigations.

Robert Keith Shepherd

The present research used both physiological and histological techniques to assess the effects of chronic intracochlear electrical stimulation on the residual auditory nerve population in cats. Stimuli consisted of charge balanced biphasic current pulses presented at 500 pps. Stimulus levels were in the range 0.5 - 0.9 mA, and 200 us per phase, and developed charge densities of 18 - 32 μC. cm⁻² geom. per phase. These stimulus levels are within the range used clinically. The animals were stimulated for periods of up to 2000 hours during which time electrically evoked auditory brainstem responses (EABRs) were periodically recorded. At the conclusion of the stimulus program spiral ganglion cell survival was assessed for both stimulated and control cochleas; comparison of the two groups showed no statistically significant difference in ganglion cell population. A number of cochleas exhibited various degrees of cochlear pathology in association with a general inflammation reaction. Severe inflammation, observed in four of the 20 cochleas examined, was attributed to the presence of infection and resulted in significant and widespread
neural degeneration. The histopathological changes were correlated with changes in the EABR input-output functions and confirmed the physiological viability of these cells.

The results of this study indicates that long-term intracochlear electrical stimulation, using carefully controlled biphasic pulses, does not adversely affect the auditory nerve population. However, widespread infection can result in severe loss of auditory nerve fibres and care must be taken in this regard during implant surgery. Finally, the correlation between cochlear histopathology and EABR recordings suggests that the EABR may be a useful physiological tool in determining auditory nerve survival in patients.

The impedance of these scala tympani electrodes were monitored throughout the chronic stimulation program and were compared with impedance data from similar electrodes chronically stimulated in inorganic saline. The changes in impedance of the in vivo stimulated electrodes generally correlated with the degree of fibrous tissue reaction adjacent to the electrode surface.

These scala tympani electrodes were examined for evidence of corrosion using a scanning electron microscope. The surface of these in vivo electrodes were compared with in vivo control electrodes and in vitro electrodes stimulated in inorganic saline using
similar stimulus parameters. The in vitro stimulated electrodes showed evidence of platinum dissolution at high charge densities (36 uC.cm\(^{-2}\) geom. per phase) and aggregate charge (270 C). Significantly, the in vivo stimulated electrodes showed no evidence of stimulus induced corrosion. Indeed, their surfaces were similar to the in vivo control electrodes. Previous in vitro electrochemical studies have demonstrated that proteins play a significant role in the inhibition of platinum dissolution. The present study has demonstrated an inhibitory effect in vivo which may be due to the presence of proteins.

The results from these studies reflect the biocompatible nature of this neural stimulator.

Finally, temporary and permanent reductions in the excitability of the auditory nerve were observed following acute stimulation at intensities and rates above the range used clinically. The extent of these changes correlated with the degree of stimulus evoked neural activity. Furthermore, these stimulus induced changes were metabolically active. These findings suggest that the changes in neural excitability were a result of long-term metabolic changes in the stimulated neural population. Moreover they indicate upper functional operating ranges for auditory prostheses using this form of stimulus regime.
ACKNOWLEDGEMENTS

There are many individuals that have contributed in many ways during the course of this research.

My supervisor, Professor GM Clark, made extensive contributions of both ideas and time. His energy and enthusiasm are infective and ensure a highly motivated environment in which to work. In addition I am grateful for the use of his laboratory facilities and his continued support of my work.

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There are a number of other individuals that deserve special thanks. Mr. JF Patrick of Nucleus Limited made valuable contributions to this work. His advice was always timely and astute, and he gave continued support and enthusiasm for the work in hand. Dr. MS Hirshorn made valuable contributions to the design of a number of the studies for which I am grateful. Mr. MT Murray and Dr. ME Houghton of CSIRO Division of Manufacturing Technology provided much help in carrying out the scanning electron microscope study, Miss B Labey provided statistical advice, Miss J Quilter of the RVEEH Library obtained many of my reprints, and Dr. E Javel generously provided the software used to generate the graphs in this thesis.

I acknowledge financial assistance from the Department of Science and Technology, Commonwealth of Australia; the National Health and Medical Research Council; and the Deafness Foundation (Victoria).

Finally, I would like to acknowledge the continued support of my wife Ursula, who provided encouragement, a stable and happy home environment, and who ensured that our children did not contribute to the final draft of this thesis.
ABBREVIATIONS

The International System of Units were used in this thesis. Symbols for these units were in accordance with the International Union of Pure and Applied Physics guidelines. Other abbreviations used in this thesis include the following:

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ABR</td>
<td>auditory brainstem response</td>
</tr>
<tr>
<td>ac</td>
<td>alternating current</td>
</tr>
<tr>
<td>A/D</td>
<td>analogue to digital converter</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CAP</td>
<td>compound action potential</td>
</tr>
<tr>
<td>Cd1</td>
<td>double layer capacitance</td>
</tr>
<tr>
<td>CF</td>
<td>characteristic frequency</td>
</tr>
<tr>
<td>CM</td>
<td>cochlear microphonic</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>COCB</td>
<td>crossed olivo-cochlear bundle</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>dB</td>
<td>decibel</td>
</tr>
<tr>
<td>DC</td>
<td>direct current</td>
</tr>
<tr>
<td>EABR</td>
<td>electrically evoked ABR</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>EDXa</td>
<td>energy dispersive x-ray analysis</td>
</tr>
<tr>
<td>EP</td>
<td>endocochlear potential</td>
</tr>
<tr>
<td>G</td>
<td>standard wire gauge</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>H2</td>
<td>hydrogen</td>
</tr>
<tr>
<td>HP</td>
<td>hydrogen atom plating</td>
</tr>
<tr>
<td>i</td>
<td>stimulus current</td>
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<td>i.m.</td>
<td>intramuscular</td>
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<td>i.p.</td>
<td>intraperitoneal</td>
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<td>Ir</td>
<td>iridium</td>
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k  kilo \((10^3)\)

m  milli \((10^{-3})\)

n  nano \((10^{-9})\)

N1  acoustically evoked auditory nerve CAP

N1_{E}  electrically evoked auditory nerve CAP

OD  outside diameter

OE  oxide evolution

OF  oxide formation

OR  oxide reduction

OsO_4  osmium tetroxide

pe  peak equivalent

pps  pulses per second

PSTH  post stimulus time histogram

Pt  platinum

R_a  access resistance

r.f.  radiofrequency

s.c.  subcutaneous

SEM  scanning electron microscope

SP  summating potentials

SPL  sound pressure level \((\text{re } 20 \text{ uPa})\)

TEM  transmission electron microscope

TTS  temporary threshold shift

u  micron \((10^{-6})\)

V_e  electrode voltage

Z_f  faradaic impedance

[Ca^{2+}]_i  intracellular calcium ion concentration

[Ca^{2+}]_o  extracellular calcium ion concentration

[K^+]_o  extracellular potassium ion concentration

[Na^+]_i  intracellular sodium ion concentration
CHAPTER ONE

INTRODUCTION

The clinical application of electrical stimulation has a long and colourful history spanning some 2,000 years. The first documented account of the clinical use of electrical stimulation was reportedly by a Roman physician in 46 A.D. He prescribed the use of the electric ray or torpedo fish (Torpedinidae) for the treatment of pain such as headache and gout (McNeal, 1977). The fish was placed on the patient's body over the site of the pain until the pain ceased. However, even in this first account, the practitioner was aware of adverse effects associated with electrical stimulation, warning that longer exposure to electrical stimulation risked the chance of a localized loss of feeling.

The modern era of electrical stimulation of excitable tissue began in the mid 1950's with the development of the heart pacemaker. The success of this device, together with a great increase in the understanding of the processes associated with the generation and propagation of nerve impulses that had occurred over the preceding 60 years, has resulted in a proliferation in the investigation of neural stimulators covering a wide range of applications.
The technological revolution in the electronics industry has meant that sophisticated, portable neural prostheses can be realized.

Within the past 10 years a great number of neural stimulators have been developed and evaluated experimentally. Some have become available clinically. These applications include stimulation of the phrenic nerve for diaphragm pacing in patients suffering from hypoventillation; stimulation designed to mimic the micturition reflex function in order to provide effective urinary bladder evacuation in patients with spinal injuries; cerebellar stimulation for the control of epilepsy and to provide some motor control in disorders associated with cerebral palsy and spasticity; electrical stimulation of the visual cortex in order to provide rudimentary visual information for blind patients; and electrical stimulation of the auditory pathway in order to provide speech cues for the profound and totally deaf.

One fundamental question that all research groups investigating neural prostheses must address is device safety. These safety considerations include biocompatibility of the implanted prosthesis; the extent of trauma associated with the surgical placement of the stimulating electrode(s); the effect of long-term electrical stimulation on the target neural population both directly and indirectly as - for example - a result
of the release of toxic electrochemical products from the electrode in response to the charge injection process, or the activation of local arteriolar sphincters which would reduce the effectiveness of the local circulatory system; and finally, an evaluation of the increased risk of infection which is associated with the implantation of any prosthetic device, and the techniques used to minimize this risk.

Like the early Roman physician, we must be aware of the adverse effects associated with electrically active prostheses and this awareness must be incorporated in good design procedures. Following well established design guidelines is, however, not sufficient to ensure design safety. Each neural stimulator must be evaluated using appropriate animal models in the site the device is intended for use. Seemingly small changes in the device design or surgical placement could lead to a previously safe device evoking an adverse tissue reaction or becoming inoperative. For example, placing a neural stimulator in a new implant site may alter the geometrical relationship between the electrode array and the target neural population in such a way that higher currents are required for effective neural stimulation. Elevated stimulus levels could place the electrode array under greater electrochemical stress. Similar effects could also be associated with attempts to stimulate smaller diameter nerve fibres as neural
threshold is inversely proportional to fibre diameter. Alternatively, a change in the site of implantation may reduce the biocompatibility of the device due, for example, to its placement in a site subjected to greater muscular movement resulting in an increased chronic inflammatory reaction. A new surgical site could also increase the risk of infection being associated with the prosthesis. For example, scala tympani electrodes used in cochlear prostheses must pass through the middle ear cavity, breaching the natural barrier between the middle ear and the cochlea. The effects of infection associated with an electrode route such as this, requires extensive investigation as the middle ear cavity is particularly vulnerable to infection. Moreover, infection tracking into the cochlea would not only significantly reduce the neural population available for electrical stimulation but could also result in serious complications including meningitis.

This thesis is primarily concerned with the assessment of intracochlear electrical stimulation on both the adjacent primary auditory nerve fibres and the metal stimulating electrode, and forms a part of the safety and efficacy studies of the Nucleus Limited Cochlear prosthesis. The physiological and histopathological effects of long-term intracochlear stimulation at stimulus levels within the range used clinically were evaluated and compared with unstimulated
control cochleas (Chapter 4). The chronically stimulated electrodes were then examined under a scanning electron microscope (SEM) for evidence of electrode corrosion (Chapter 5). The surface of these electrodes were compared with both in vivo control electrodes and electrodes stimulated in inorganic saline. In addition, electrode impedance data, which was collected periodically during the chronic stimulation program, was evaluated and impedance changes were correlated with the degree of fibrous tissue associated with the electrode array (Chapter 5). Finally, the response of the auditory nerve was physiologically monitored following acute periods of intracochlear electrical stimulation at stimulus intensities and rates that ranged from clinical levels to levels that were significantly greater than the maximum levels used clinically (Chapter 6). These acute physiological changes were correlated with ultrastructural changes by examining these cochleae under a transmission electron microscope (TEM). These results provided some indication as to the maximum safe stimulus regimes for cochlear prostheses and implicates significant metabolic changes in the excitable tissue as a potential neural damage mechanism.

Although studies investigating other safety issues associated with cochlear prostheses are outside the scope of this thesis, many of these issues have been addressed by investigators in this laboratory. These
include the development of a surgical protocol (Clark et al., 1984), a protocol designed to minimise the risk of infection associated with the surgical placement of the device (Clark et al., 1980), an evaluation of the extent of trauma associated with the insertion of the electrode array (Shepherd et al., 1985; Franz et al., in press) the effects of middle ear infection on cochlear prostheses (Clark and Shepherd, 1984; Franz et al., 1984), and an evaluation of the biocompatibility of the prostheses (Clark et al., 1983; Shepherd et al., 1984).
2.1 Introduction.

Since this thesis is primarily concerned with the electrophysiological and histopathological response of the cochlea to intracochlear electrical stimulation, the anatomy and physiology of the external and middle ear will only be considered briefly. The anatomy and physiology of the mammalian cochlea, including neuroanatomy, cochlear dynamics and cochlear electrophysiology, will be described in some detail. The discharge patterns from single auditory nerve fibres and the central pathway of the ascending auditory system will be briefly discussed. Finally, a review of the physiological basis of electrical stimulation of neural tissue in general, and the auditory nerve in particular, shall be presented.

2.2 Gross Anatomy of the Peripheral Auditory System.

The basic features of the anatomy of the mammalian ear are illustrated in the schematic diagram of the human ear (Fig. 2.1). The gross anatomy of the peripheral auditory system can be subdivided into three parts: 1) the outer ear, consisting of the auricle or
pinna and the external auditory meatus (ear canal); 2) the middle ear containing the tympanic membrane, the middle ear cavity and the ossicular chain; and 3) the cochlea or inner ear which contains the delicate sensory cells capable of transducing mechanical motion into coded neural impulses.

Figure 2.1  Schematic diagram of the gross anatomy of the peripheral auditory system. (from Brodel, 1946)
The primary function of the external ear is one of protection. In the human at least half the length of the external auditory meatus contains a bony wall continuous with the temporal bone, offering protection to the delicate middle ear structures. Additional protection is afforded by the epithelial lining of the external meatus. This lining contains numerous wax secreting cells and hairs which help prevent foreign matter interfering with the tympanic membrane.

The external ear also has some important acoustic properties. The pinna serves as an efficient collector and amplifier of sound (Stebbins, 1983), and the external ear tends to act as an acoustic funnel. The resonance characteristics of the external auditory meatus have been measured in both human (Wiener and Ross, 1946) and the cat (Wiener et al., 1966). Both studies have shown a pressure resonance at the tympanic membrane for the mid-frequency range centred at approximately 3 kHz (Fig. 2.2). The pressure amplification in humans was found to be approximately 10 dB, considerably lower than that measured in the cat (Fig. 2.2). This is probably due to the differences in geometry of the external ear between species.

The middle ear is an airborne cavity bounded laterally by the tympanic membrane and medially by the cochlea. This cavity contains the ossicular chain; the malleus, incus, and stapes, that convey the
Figure 2.2  Resonance characteristics from free field to the tympanic membrane in the cat. This graph represents data from five animals.
(from Wiener et al., 1966).
vibratory motion of the tympanic membrane to the oval window of the cochlea. The middle ear cavity is continuous with the external environment via the eustachian tube, thus enabling the pressure on both sides of the tympanic membrane to be equalized.

The tympanic membrane is a cone shaped membrane held under tension by its fibrous attachment to the bony external canal and the tensor tympani muscle. The membrane consists of three layers; two layers are continuous with the epithelial lining of the external and middle ears, and a fibrous middle layer that consists of fibres both radially and concentrically arranged. It is this layer that provides much of the mechanical properties of the tympanic membrane.

In terrestrial mammals, the ossicular chain consists of three distinct bones attached to one another by ligaments (Fig. 2.1). The long process (manubrium) of the malleus is attached to the middle bone of the chain, the incus, which in turn is attached to the head of the stapes. The footplate of the stapes is attached to the oval window of the cochlea via an annular ligament. The tympanic membrane functions as a resonator in response to sound waves from the external canal, motion is transferred through the ossicular chain to the stapes footplate whose movement is similar to that of a hinged door.
The motion of the ossicular chain can be regulated by the contraction of two small skeletal muscles; the tensor tympani is attached to the long process of the malleus and the stapedius muscle to the stapes. Although it was once thought that these muscles increased the transmission of weak sound vibrations, it is now recognised that their function is to protect the delicate middle and inner ear structures from excessively loud noise. Contraction of these muscles (the tympanic reflex) results in a stiffening effect on the ossicular chain, reducing the transmission of loud sounds. A 40 dB attenuation of the middle ear transmission function has been measured in the cat (Wiggers, 1934).

Careful matching is required to ensure that the vibratory motion of the incident airborne sound waves are transferred efficiently to the fluid filled spaces of the inner ear. Impedence matching is achieved by two mechanisms. First, force manipulation in the middle ear via the compound lever action of the malleus and incus and second, pressure amplification resulting from differences in areas of the tympanic membrane and oval window.

In the anaesthetised cat and human cadaver temporal bones, the middle ear transmission function has been found to be linear to sound pressures of up to 100–130 dB SPL although it probably does not operate
linearly above 70 dR SPL, the level of the tympanic reflex, a non-linear mechanism (Dallos, 1973; p 91). The middle ear transfer function is frequency dependent with characteristics similar to a low pass filter having a cutoff frequency of approximately 1200 Hz (Guinan and Peake, 1967).

2.3 Gross Anatomy of the Inner Ear.

The inner ear or cochlea is contained within the bony labyrinth which also contains the vestibule and semicircular canals of the vestibular system. These structures are illustrated in Fig. 2.3. The insert in the figure shows the anatomical position of the bony labyrinth within the human skull.

The cochlea is a fluid filled tubular structure that spirals around a central axis called the modiolus. The modiolus supports the spiral cochlea and contains the spiral ganglion cells, the perikaryon of the primary auditory neurones. These structures are illustrated in mid-modiolar view in Fig. 2.4.

The number of turns and the length of the cochlea spiral vary among species. Some examples are given in Table 2.1. Cochlear length and number of turns appear to be unrelated to an animal's audible frequency range (Stebbins, 1983; p 84).
Figure 2.3 Schematic representation of the human bony labyrinth. The insert shows the anatomical position of the left and right bony labyrinth within the human cranium. (from Schuknecht, 1974).
Figure 2.4  Mid-modiolar section of guinea pig cochlea showing the three cochlea scalae, the auditory nerve, and the arterial and venous supplies within the modiolus. (from Axelsson, 1968).
As can be seen from Fig. 2.4, the spiral cochlea is divided along its length into three distinct compartments or scalae. The scala tympani is formed below the cochlear partition or basilar membrane, the scala vestibuli is formed above this partition. These two compartments communicate with one another at the helicotrema which is located at the apex of the cochlea. These compartments contain perilymph, a fluid similar in chemical composition to cerebrospinal fluid (CSF). A smaller third compartment, the scala media, is located between these larger scalae, being bounded by the basilar membrane and Reissner's membrane. The scala media contains endolymph and is continuous with the saccule, utricle and the semicircular canals of the vestibular system.
2.4 Fine Anatomy of the Mammalian Cochlea.

The sensory cells of the cochlea are contained within the organ of Corti, a complex structure that is attached to the basilar membrane at its base and the tectorial membrane at its apex, and extending along the entire length of the cochlear partition. Fig. 2.5 illustrates the relationship of the organ of Corti to the cochlear scalae.

The structural framework of the organ of Corti is provided by the inner and outer pillar cells (rods of Corti), the inner phalangeal cells, Claudius's cells and the border cells. This framework primarily supports the sensory hair cells and the dendritic processes of the auditory nerve fibres. The space bordered by the reticular lamina, Hensen's cells, the basilar membrane and the spiral limbus contains Cortilymph, a fluid of similar ionic composition to perilymph (Engstrom, 1960). Fig. 2.6 provides a more detailed illustration of the organ of Corti.

The sensory hair cells are arranged into two types, one on either side of the pillar cells. On the lateral side, bathed in Cortilymph, are three to four rows of outer hair cells. These cells are supported at their apex by the reticular laminar and their base by Deiters' cells (outer phalangeal cells). The outer hair cells are cylindrical in shape having a diameter of approximately 10 um and a length that can vary from 30 to
Figure 2.5 Diagrammatic sketch showing the anatomical structures of the mammalian cochlear duct.
(from Davis, 1962).
70 μm, increasing in length from base to apex. Each cell contains a rigid cuticular plate at its apex from which protrude 50 to 150 stiff stereocilia arranged in 3 to 6 rows to form a W-like pattern. These stereocilia have a typical diameter of 0.15 μm and average 6 μm in length, and like the outer hair cells to which they are attached, the length of the stereocilia increases from cochlear base to apex.

The tips of the longest stereocilia from each outer hair cell extend to the tectorial membrane (Kimura, 1966; Spoendlin, 1966), an acellular structure attached to the spiral limbus on the modiolar side of the cochlea and possibly the cells of Hansen on the lateral side of the cochlea (Kronester-Frei, 1979). The apical end of the outer hair cells have a rich supply of mitochondria, suggesting a region of high metabolic activity. Synaptic junctions are located in the lower portion of the cell.

Inner hair cells lie on the modiolar side of the pillar cells and are completely surrounded by supporting cells. These cells are slightly smaller and more rotund than the outer hair cells, and, unlike the outer hair cells, there is little difference in size of the inner hair cells along the cochlear spiral (Iurato, 1967; p 27). Forty to 60 stereocilia protrude from the cuticular plate, these cilia are smaller and coarser than those on the outer hair cell. The question as to there
Figure 2.6  Detailed diagram of the organ of Corti and its afferent and efferent innervation.
(from Bodian, 1983).
attachment to the tectorial membrane has still to be resolved (Ross, 1974; Hoshino, 1976) Like the outer hair cells, inner hair cells have a rich supply of mitochondria in the apical portion of the cell, however, in contrast with outer hair cells, these cells have synaptic junctions extending over two-thirds of the length of the cell.

2.5 Cochlear Neuroanatomy.

There are three known components of cochlear innervation, the afferent system, the efferent system and the autonomic system. Of these only the afferent system is known in any detail.

There are approximately 50,000 afferent auditory nerve fibres in the cat, and 30,000 in human. The ganglion cells are located peripherally within Rosenthal's canal, a space that follows the spiral course of the cochlea and is contained within the osseous spiral limina. In the cat and other small mammals two types of ganglion cells are found within the spiral ganglion (Spoendlin, 1972; Ryan and Schwartz, 1983); the most common (Type I) represent 90 to 95% of the spiral ganglion, and are characterized by a large myelinated cell body with a round nucleus and prominent nucleolus. In contrast, Type II ganglion cells are approximately half the size of the Type I cell with a lobulated nucleus and an insignificant nucleolus. Type
II cells are unmyelinated in most species, and typically represent 5 to 10% of the spiral ganglion population.

Peripheral axones (dendrites) from both Type I and II ganglion cells project radially towards the organ of Corti. Dendrites from Type I cells lose their myelin sheath as they pass through an opening in the osseous spiral lammina called the habenula perforata. Approximately 10 to 20 fibres pass through each habenula opening and continue to follow a radial path towards the organ of Corti. The afferent and efferent innervation patterns for the mammalian organ of Corti are illustrated in Figs. 2.6 and 2.7.

Ninety to 95% of all fibres lead directly to the nearest inner hair cell where they form a synaptic junction. The inner hair cell is therefore innervated by approximately 20 fibres over a large area of the cell body. The remaining five to 10% of fibres pass between the pillar cells and project towards the outer hair cells along the floor of the tunnel of Corti. During their passage across the tunnel of Corti, these fibres tend to move in a basalward direction (Lorente de No, 1933). This basalward movement becomes significant once the fibres pass between Deiter's cells. Each fibre will typically project 0.6 mm basally prior to innervating approximately 10 outer hair cells with collaterals branching from the main fibre.
Because it is not possible to identify Type I and Type II dendrites within the organ of Corti, there has been considerable debate concerning the innervation patterns of these two types of neurones. However, studies based on the selective degeneration of Type I neurones following the sectioning of the auditory nerve, has demonstrated that Type II neurones provide exclusive innervation to the outer hair cells (Spoendlin, 1972). More recently, intracellular microelectrode injection techniques have been used to demonstrate the projection of Type I spiral ganglion cells to the inner hair cells of the organ of Corti (Robertson, 1985).

Axons of both Type I and Type II spiral ganglion cells are known to project to the cochlear nucleus, and this nucleus is therefore directly connected to both the inner and outer hair cells (Jones et al., 1984).

Ganglion cell morphology appears to be considerably different in human compared with the small mammals such as the cat (Ota and Kimura, 1980). Although there are two cytologically distinct spiral ganglion populations - large and small - both populations consist of myelinated and unmyelinated cells, with the great majority (94%) being unmyelinated. The functional significance of these morphological findings is not clear. The innervation patterns of the organ of Corti are similar to those found with the smaller mammals. A large majority of ganglion cells innervate inner hair
cells while only 10 - 20% of ganglion cells innervate outer hair cells (Nomura, 1976; Ota and Kimura, 1980).

The divergent innervation pattern of the inner hair cells contrasts with the convergent pattern of the outer hair cells and indicates a fundamental difference in their functional mechanisms.

Although evidence for the existence of an efferent cochlear nerve supply was first given in 1942 (Rasmussen, 1942), its functional significance is still poorly understood (Spoendlin, 1973; Robertson, 1985). The efferent supply descends from the auditory cortex through several relays, and finally enters the basal region of the cochlea via the olive-cochlear bundle. This bundle of approximately 500 fibres spirals apicalward as the intraganglionic spiral bundle contained within Rosenthal's canal.

Efferent fibres diverge radially from the intraganglionic spiral bundle and branch to approximately 3000 fibres as they enter the organ of Corti via the habenula perforata. Under the inner hair cells the efferent fibres continue their apicalward spiral as the internal spiral bundle, with a number of fibres providing collaterals which terminate on the afferent fibres innervating the inner hair cells. The majority of fibres, approximately 8,000, cross the tunnel of Corti as the upper tunnel radial fibres, and further branching takes place as they approach the outer hair cells.
Figure 2.7 A schematic summary of the innervation pattern of the organ of Corti of the cat. IHC, inner hair cell; OHC, outer hair cell SG, spiral ganglion.
(from Spoendlin, 1973).

Approximately 40,000 efferent nerve endings contact the outer hair cell bodies directly, with the greatest innervation density in the basal turn and substantially less apicalward.

2.6 Ultrastructure of Primary Auditory Neurones.

The two cell types that constitute the spiral ganglion show distinct and contrasting ultrastructural detail. The perikaryon of the Type I spiral ganglion cell has a granular cytoplasm containing many ribosomes and granular endoplasmic reticulum (Nissl substance), which tend to be clumped around the nucleus (Rosenbluth,
towards the axon hillock where mitochondria and filamentous structures - neurotubules and the finer neurofilaments - are found in greater numbers. These filamentous structures appear unaligned close to the perikaryon, however, they become increasingly oriented with the longitudinal axis of the axon distally (Romand and Romand, 1984).

The nucleus of this cell type is relatively large and round. It is generally lighter in appearance than the cytoplasm, containing fine evenly dispersed chromatin. The nucleolus is an extremely electron dense spherical structure containing dense ribosome like granules and closely packed fine filaments (Rosenbluth, 1962; Ylikoski and Galey, 1984; Fig. 2.8).

Unlike the majority of spinal and sensory ganglion, the perikaryon of the Type I spiral ganglion and all perikaryon of Scarpa's ganglion (the vestibular ganglion) are enveloped by a thin myelin sheath of typically 1 - 6 lamellae (Spoendlin, 1972; Ryan and Schwartz, 1983; Ylikoski and Galey, 1984). This thin myelin sheath envelopes the cell body and axon hillock to the first node of Ranvier. The axon and dendrite possess a considerably thicker myelin sheath.

Schwann cells associated with the Type I spiral ganglion generally have a large ovoid shaped nucleus which contains dense chromatin. The dark cytoplasm
Figure 2.8  Electron micrograph showing a Type I spiral ganglion perikaryon from the basal turn of a cat cochlea. The cytoplasm is rich in ribosomes, granular endoplasmic reticulum (E), and mitochondria. Occasional Golgi body (G) and dark inclusion bodies (D) are also present. The lighter, more filamentous nucleus contains an electron dense nucleolus. A thin myelin sheath (M) envelops the perikaryon. x12200

Figure 2.9  Electron micrograph of a cat auditory nerve axon. The large axoplasm contains neurotubules and neurofilaments. The axon is surrounded by a thick myelin sheath. x20000
contains mitochondria, granular endoplasmic reticulum and ribosomes (Romand and Romand, 1984). The nucleolus is less conspicuous than the nucleolus of the Type I ganglion cell.

The axonal and dendritic processes emerge from opposite sides of the perikaryon illustrating the true bipolar nature of these cells. Both processes consist of a large axoplasm containing neurofilaments and neurotubules, and are surrounded by a thick myelin sheath typically containing up to 50 lamellae (Fig. 2.9). The central process is 4 - 6\,\mu m in diameter and shows uniformity throughout the auditory nerve. The peripheral process is somewhat smaller, typically 2\,\mu m within the osseous spiral lamina, although its diameter is significantly reduced as the fibre passes close to physically important structures within the organ of Corti and the habenula perforata (Spoendlin, 1984). The peripheral process of the Type I ganglion cell innervates the inner hair cell, forming synaptic complexes over a large region of the inner hair cell. Synaptic bars and an accumulation of synaptic vesicles are present within the inner hair cell adjacent to the complex (Spoendlin, 1984).

The Type II spiral ganglion cell perikaryon are smaller and possess a lighter cytoplasm than the Type I ganglion cell. The cytoplasm contains more filamentous material and fewer ribosomes, granular endoplasmic
reticulum and mitochondria. The cell nucleus is typically eccentric and lobulated (Spoendlin, 1972; Romand and Romand, 1984). Unlike Type I ganglion cells, these cells are unmyelinated, although they are surrounded by a Schwann cell process (Romand and Romand, 1984). The central and peripheral processes of the Type II spiral ganglion often emerge from the same side of the cell body, these cells are therefore not true bipolar neurones. Moreover, their processes always have a smaller axoplasm than the myelinated fibres, although they generally have a greater density of neurotubules. The peripheral process of the Type II ganglion cell makes direct contact with the outer hair cell although their synaptic complexes are not prominent (Spoendlin, 1984).

The significant ultrastructural differences between Type I and Type II spiral ganglion cells reflect the functional differences between these two cell types. Although there are morphological differences between human spiral ganglion and the spiral ganglion of small mammals on the basis of perikarya myelinization, the two populations of human spiral ganglion show some cytoplasmic details that closely resemble the two populations in smaller mammals. The majority of human spiral ganglion cells possess a cytoplasm rich in ribosomes, rough endoplasmic reticulum and mitochondria. Moreover, these cells contain a rounded nucleus rich in chromatin, and containing a distinct,
compact nucleouls. A minority of human spiral ganglion contain a large number of neurofilaments at the expense of both rough endoplasmic reticulum and ribosomes (Kimura et al., 1979; Ota and Kimura, 1980; Romand and Romand, 1984). On the basis of these ultrastructural findings it is possible that the majority of human spiral ganglion cells (both myelinated and unmyelinated) have a similar functional role as the Type I spiral ganglion. Moreover, the smaller human spiral ganglion cells may have similar functional roles as the Type II ganglion cells found in the smaller mammals.

2.7 Cochlear Dynamics.

The frequency specificity of the basilar membrane was first recognised over one hundred years ago when Helmholtz introduced his resonance-place theory (Helmholtz, 1885). However it was not until the middle of this century that the experimental work of von Bekesy clearly demonstrated that a given location along the basilar membrane is maximally sensitive to a specific frequency. von Bekesy optically measured the amplitude and phase characteristics along the basilar membrane of human cadaver cochleas at a number of frequencies (Fig. 2.10). These results indicated that the distance from the helicotrema to the position of maximum displacement along the basilar partition is nearly proportional to the logarithm of the frequency of acoustic stimulation.
There is considerable discrepancy, however, between the frequency tuning characteristics of the basilar membrane (von Bekesy, 1960) and the high degree of frequency selectivity of single auditory nerve fibres (Kiang et al., 1965). Fig. 2.11 shows the highly tuned frequency response characteristics of several single auditory nerve fibres compared with the frequency response of the basilar membrane obtained from guinea pig cadaver preparations (Evans, 1972).

A second filter was postulated to account for the large discrepancy in tuning characteristics between the basilar membrane and single auditory nerve fibres (Evans and Wilson, 1975). The discovery that the inner hair cell receptor potential showed similar frequency selective properties as single auditory fibres indicated that the frequency selectivity was present in the mechanical input to the inner hair cell (Russell and Sellick, 1978). Recent measurement of basilar membrane motion in living animals using the Mossbauer technique (Sellick et al., 1982) and laser interferometry (Khanna and Leonard, 1981) have produced tuning characteristics similar to those obtained for single auditory nerve fibres. These studies have also demonstrated the sensitivity of the basilar membrane tuning properties to mechanical and physiological insult and therefore explain the broad tuning responses obtained by von Bekesy using his cadaver preparations.
Figure 2.10 Patterns of vibration amplitudes of the basilar membrane in a human cadaver cochlear at several frequencies. Horizontal axis is distance from stapes. Solid lines represent experimental data, dashed lines are von Bekesy's extrapolations.
(from von Bekesy, 1960; p 448).
Figure 2.11 Frequency-threshold curves for single auditory nerve fibres of the guinea pig. Dotted lines show the more broadly tuned basilar membrane response curves from guinea pig cadaver preparations (von Bekesy, 1960). These basilar membrane tuning curves are positioned arbitrarily on the intensity scale.

(adapted from Evans, 1972).
These results have generated a number of models that attempt to explain the sharp basilar membrane filter characteristics and the physiological vulnerability of the responses. All models place considerable importance on the role of the outer hair cells, as the sensitivity of the mechanical response measured at the basilar membrane has been correlated with the condition of the outer hair cells (Khanna, 1983). The majority of models propose a physiologically active process that sharpens the basilar membrane vibration characteristics, but which is physiologically vulnerable (e.g. Davis, 1983). These active models also explain the origin of the cochlear echo first described by Kemp (Kemp, 1978). In contrast to these active models, Zwislocki (Zwislocki, 1983) proposes a second mechanical resonator involving the outer hair cells and the coupling of their stereocilia to the tectorial membrane. It is also possible that the efferent system may effect the tuning properties of the basilar membrane, as the outer hair cells are well innervated by efferent fibres from the crossed olivocochlear bundle (COCB) (Sellick et al., 1982). Indeed, recent experimental evidence has shown that inner hair cell responses can be altered by efferent COCB stimulation (Mountian, 1980; Brown et al., 1983). These alterations in the inner hair cell response are probably a result of a mechanical interaction between the outer hair cell stereocilia and the tectorial membrane.
Although the mechanism producing the sharp basilar membrane tuning properties is by no means clear, experimental work by von Bekesy as early as 1951 (von Bekesy, 1951) has shown that the sensory process taking place in the cochlea is not just a simple transformation of energy, but a complicated process in which there is controlled release of biological energy resulting in amplification.

### 2.8 Cochlear Electrophysiology.

Although the mechanical tuning characteristics of the basilar membrane (Khanna and Leonard, 1982; Sellick et al., 1982), and the significant role of the organ of Corti (Zwislocki, 1983; Davis, 1983) in this process is now well recognised, the transduction of mechanical motion into action potentials along the auditory nerve is not well understood. However, the discovery and recording of various potentials within the cochlea has provided an experimental foundation from which theories describing the transduction process have developed.

Two classes of cochlear potentials have been recognised within the mammalian cochlea; resting potentials and stimulus related potentials.

The DC resting potentials were first investigated in systematic detail by von Bekesy (von Bekesy, 1951). Table 2.2 gives more recent results of measurements of these potentials.
Table 2.2 Average DC Resting Potentials within the Mammalian Cochlea.

<table>
<thead>
<tr>
<th>scala tympani</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>organ of Corti (intracellular ?)</td>
<td>-80 mV</td>
</tr>
<tr>
<td>scala media</td>
<td>+100 mV</td>
</tr>
<tr>
<td>scala vestibuli</td>
<td>0 mV</td>
</tr>
</tbody>
</table>

The positive endocochlear potential (EP) of the scala media and the negative potential within the organ of Corti, are thought to contribute to the biologically active transduction process (Davis, 1968).

The generation of EP is a physiologically active process, anoxia results in a severe reduction of this potential (Davis et al., 1958). Tasaki and Spiropoulos (1959), utilizing differential recording techniques identified the stria vascularis as the generator of the EP. Endolymph does not contribute to the EP, as a potential of +4 mV has been recorded in the utrical although this space is also occupied by endolymph (Smith et al., 1958). Thus it would appear that the EP is generated by an active process localized to the stria vascularis.

Negative potentials of between 70 and 100 mV have been recorded from electrodes placed within the organ of Corti. There is, however, considerable debate as to the site of these potentials. Lawrence maintains that the
DC potential is that of Cortilymph (Lawrence, 1967), while Dallos contends that the DC potential is a result of intracellular recordings from cells within the organ of Corti (Dallos, 1973). Dallos objects to such a large negative DC potential associated with organ of Corti fluid on theoretical grounds, maintaining that hair cells and their dendrites would be permanently depolarized and would therefore be unable to initiate action potentials via the classical active membrane process.

There are three stimulus induced cochlear potentials currently recognised; the cochlear microphonic (CM), summation potentials (SP) and the compound action potential (CAP). The CM and SP are both generated by similar processes within the organ of Corti. The CM is an ac potential with a waveform similar to the acoustic stimulus. The CM consists of at least two components; the first order CM is generated at the upper surface of the organ of Corti (Tasaki et al., 1954), and is sensitive to anoxia. Reissner's membrane is thought to be a second order CM generator. Although this CM source is a smaller contributor, it is less sensitive to anoxia and is thought to be the active generator of CM observed in fresh cadavers. Davis and colleagues (Davis et al., 1958) showed that the CM is best correlated with the integrity of the outer hair cells, the stereocillia of which are now generally accepted as the site of the primary CM production.
The mechano-physiological processors involved in the generation of an action potential are thought to include an active physiological process spatially and temporally triggered by incident mechano-acoustic energy. As CM (and SP) are mechanically induced electrical phenomenon that indicate the initiation of the transduction process, understanding the nature of their generation is fundamental to the understanding of the entire transduction process.

Although there are several theories that attempt to explain CM production the most widely accepted is Davis's resistance modulation hypothesis (e.g. Davis, 1968). This hypothesis proposes that two series biological batteries maintain a steady current flow across the organ of Corti (Fig. 2.12). These biological batteries are the EP and the negative DC polarization potential of the hair cells. During sound stimulation outer hair cell stereocillia connected to the tectorial membrane are subject to radial shearing forces due to the relative movement of the basilar membrane and the tectorial membrane. These forces deform the top of the hair cells and therefore result in changes in the electrical resistance, producing changes in the current flow across the outer hair cells. Such modulation of current is thought to be the mechanism of CM production.
Several experimental studies have produced results that support this theory. von Bekesy demonstrated that radial displacement of the hair cells is the most effective way of evoking a CM response (von Bekesy, 1960). Honrubia and Ward (1969) reverse polarized the EP by placing electrodes in the scala tympani and scala vestibuli of an oxygen deprived cochlea. These investigators recorded CM (and SP) as they increased the negative polarization of the EP (Fig. 2.13). They found that CM reduced in magnitude with increasing negative polarization until, at an EP of -57 mV (approximately the resting intracellular hair cell potential) the CM could not be elicited. Significantly, further negative polarization of the EP resulted in an increase in the CM (and SP), however in reverse phase.

The SP is a stimulus induced DC potential superimposed on the CM. This group of potentials are believed to originate from a number of sites within the organ of Corti, and like the CM they are sensitive to anoxia and externally applied polarization currents. An SP has recently been recorded during intracellular recordings of inner hair cells (Russell and Sellick, 1978). These investigators suggested that the SP may play an active role in auditory nerve excitation.
The Davis resistance modulation hypothesis for hair cell excitation in the cochlea. The stria vascularis maintains the positive endocochlear potential, and the lateral cell membrane maintains a negative DC polarization within the hair cell. The current across the organ of Corti can be modulated by a variable resistance situated at the outer hair cell, and sensitive to the apical surface of the hair cell. (from Davis, 1968).
Figure 2.13 Recordings of CM responses at various degrees of negative EP polarization.

The CAP is recorded from similar sites as those used in the recording of the CM. The CAP is the summed response of a great number of neurones firing in synchrony, and therefore is best elicited using a stimulus with a rapid onset such as a click or a short rise-time tone pip. The magnitude of the CAP is
proportional to the number of excited nerve fibres. The CAP is often used as a sensitive monitor of the physiological status of the experimental cochlea. At low intensities, and using appropriate tone pip stimuli, the CAP from discrete neural populations along the basilar partition may be successfully monitored. It is common to observe latency differences of up to 4 ms between basal and apical neural populations as a result of the delay in firing of apical neurones due to the travelling wave movement along the basilar membrane. Estimates of travelling wave velocity have been made on the basis of these CAP latency differences (Eggermont, 1976). At higher stimulus intensities there is a greater proportional contribution of more basal fibres to the CAP.

2.9 Discharge Patterns from Single Auditory Nerve Fibres.

The use of microelectrodes to record the activity of single auditory nerve fibres has been in progress for over forty years (Galambos and Davis, 1943). Investigators using this technique have contributed a significant understanding to the auditory encoding process. Furthermore, these data have also contributed to the knowledge of the transduction process.

The discharges of auditory nerve fibres obeys the all-or-none law, suggesting that no information is carried by the amplitude of the spikes (Kiang et al., 2-37).
1962). All auditory nerve fibres show varying amounts of spontaneous activity in the absence of acoustic stimulation (Kiang et al., 1962). This spontaneous activity has been shown to be significantly reduced in pathological cochleas (Kiang et al., 1965; Evans, 1972). In animals with normal cochleas, the spike rate increases above the spontaneous activity during acoustic stimulation.

All auditory nerve fibres exhibit highly tuned frequency response characteristics as measured by so-called frequency tuning curves (Fig 2.11). These curves are obtained by measuring the intensity of pure tones for frequencies at which the spike rate of a particular unit is increased, above the spontaneous rate, to a certain defined threshold. The characteristic frequency (CF) for each fibre is that frequency at which the tone threshold is lowest, and corresponds closely to the most sensitive frequency of the basilar membrane at the point where the fibre innervates the cochlea (Robertson and Manley, 1974; Liberman, 1982). Thus, the CF of auditory nerve fibres varies along the cochlear partition in a manner similar to the mechanical response of the basilar membrane. This tonotopic organization of the cochlea and its centrally projecting neurones forms the basis of the so-called place theory of frequency encoding.
The firing patterns of single units with CF less than 5 kHz, to acoustic click stimuli are characteristic of that unit when the neural discharges are examined using a post-stimulus time histogram (PSTH). Such responses show a series of peaks separated by intervals equal to 1/CF (Fig 2.14; Kiang et al., 1965). For units with CF above 5 kHz there is usually one short-latency peak in the PSTH.

The discharge rate of a single auditory fibre in response to tonal stimuli increases above the spontaneous rate until a maximum rate is reached. Both the maximum discharge rate and the intensity of the tone at which the rate is maximum differs greatly among fibres (Kiang et al., 1965), however, each unit has a restricted dynamic range of typically 20 to 30 dB (Kiang, 1968; Evans 1975), significantly less than the psychophysical dynamic range. This fundamental observation indicates that a second neural coding scheme must be considered to account for the ability to discriminate pitch at high stimulus intensities.

In addition to the frequency selectivity of auditory nerve fibres, a number of investigators have examined the temporal response of individual fibres to acoustic stimuli (Kiang et al., 1965; Rose et al., 1971; Young and Sachs, 1979; Javel, 1981). These studies have shown that for low stimulus frequencies (< 5 kHz), the discharge rate of a particular neurone will depend on the
Figure 2.14 Post stimulus time histograms of responses to acoustic clicks from 18 units obtained in a single cat. Note the periodicity of the response for units with CF less than 5 kHz. (from Kiang et al., 1965).
intensity and frequency of the stimulus and the CF of the fibre, however, the time structure of the response is determined solely by the stimulus waveform. This so-called phase-locking of the unit activity to the stimulus waveform has been demonstrated for pure tones (Kiang et al., 1965), complex waveforms (Rose et al., 1971; Javel, 1981; Fig 2.15), and in steady state vowels where the formant frequencies dominated the temporal response patterns of the unit (Young and Sachs, 1979). Significantly, these temporal responses appear stable over a broad range of stimulus intensities. This temporal encoding of pitch forms the basis for the volley theory of pitch perception.

It is now generally held that both the place and volley principles contribute to the encoding of stimulus frequencies over the speech frequency range ( < 5 kHz), while the place theory is accepted as the means by which high frequency stimuli are processed (Whitfield, 1970).

Intensity encoding is carried fundamentally by the total number of neuronal discharges. Although, as previously described, individual units obtain a maximum spike rate after a very limited dynamic range, the total discharge rate increases with stimulus intensity as more fibres are recruited.
Figure 2.15  Period histograms for a fibre responding to a complex periodic acoustic stimulus. The two primary waveforms have been successively raised in 10 dB steps over a 50 dB range.
(from Rose et al., 1971).

2-42
2.10 Central Auditory Pathways.

A thorough discussion of the central auditory pathway is outside the scope of this thesis; the subject will only be briefly considered here.

The ascending auditory pathway consists of a complex interaction of nuclei between the cochlea and the cortex, and is represented schematically in Fig. 2.16. Although every auditory region of the central nervous system is tonotopically organized (Clompton et al., 1974), the function of cells within higher nuclei appear to become more specialized, for example cells that are responsible for inhibitory and binaural activity.

2.11 Electrical Stimulation of Neural Tissue:

Physiological Considerations.

The bioelectric generators responsible for the propagation of the action potential have their origin in the active ionic gradients maintained between the neural membrane and its external environment. This active gradient is maintained at an intracellular resting potential of \(-70\) to \(-100\) mV with respect to the extracellular environment. The ionic transport mechanism responsible for this ionic gradient includes a passive and a metabolically active process.

The propagation of an action potential requires a small, localized hypopolarization of the membrane potential, reaching some critical value much lower than
Figure 2.16  Schematic diagram of the principle ascending connections of the mammalian auditory system with respect to the auditory nerve on the right side. Axons numbered 1 and 2 project from bilaterally innervating nuclei. (from Harrison and Howe, 1974).
the resting membrane potential. Once the membrane has been hypopolarized to this critical value (typically -55 mV) there is an active change in membrane permeability resulting in a rapid transportation of ions in both directions across the membrane, giving rise to the classical action potential overshoot. The action potential is self-propagating, moving along the neurone as a wave of depolarizing voltage. Moreover, the action potential is an all-or-none phenomenon as depolarization only occurs once the cell membrane is hypopolarized to some critical potential.

Electrical currents applied to neural tissue are capable of lowering the resting membrane potential sufficiently to initiate an action potential. In order to hypopolarize the cell membrane, a stimulus current applied in a direction so as to reduce the resting membrane potential is required. This can most simply be achieved by placing a cathode close to the neurone (Fig. 2.17a). The flow of current from the neurone to the cathode results in a localized region of hypopolarization adjacent to the electrode. If the hypopolarization is sufficient then an action potential will be generated, and will be propagated in the normal manner (Iggo, 1978). If the cathode in this example is replaced with an anode, current will flow in the opposite direction.
(Fig. 2.17b), therefore resulting in hyperpolarization of the membrane potential adjacent to the electrode, and thus making the excitation of the neurone more difficult. Monopolar anodal stimuli do, however, evoke action potentials. Although there is an inward flow of current towards the neurone adjacent to the electrode, there is outward flow at nodes of Ranvier some distance from, and on both sides of the stimulating electrode. If the current flow at these sites is sufficient then action potentials — both orthodromic and antidromic — will be generated. The location of the sites at which these action potentials are generated will depend on the electrode geometry and the magnitude of the stimulus current. Measurement of monopolar anodal stimuli show stimulus thresholds of two to three times greater than that of monopolar cathodal stimuli (Rank, 1975).

Although bipolar electrical stimulation of neural tissue introduces further considerations such as inter-electrode spacing and electrode-neurone alignment, the underlying process of action potential generation is the same. Hence, an examination of action potential generation using bipolar electrodes will not be considered in this review.
Figure 2.17  Current flow in a neurone when current is passed from a nearby monopolar electrode. a) from a cathode; b) from an anode. The membrane is depolarized when there is an outward flow of current across the membrane. (from Rank, 1975).
2.12 Electrical Stimulation of the Auditory Nerve: 
Physiological Considerations.

2.12.1 Single unit studies.

When hair cells are missing many of the essential 
details of the auditory neural coding process are 
eliminated, including the spatial and temporal 
distribution of frequency. The cochlear prosthesis is 
designed to artificially stimulate the primary auditory 
nerve fibres in order to provide rudimentary speech cues 
to the profoundly and totally deaf. The question as to 
which speech parameters are required in a successful 
stimulus coding strategy is fundamental to cochlear 
prosthesis development.

Knowledge of stimulus coding in the normal 
auditory nerve provides a basis for the design of a 
prosthesis and its coding scheme, however, as discussed 
previously, there remains considerable uncertainty as to 
the response of the auditory nerve to complex stimuli 
such as speech, and the manner in which this coded 
information is processed centrally (Kiang and Moxon, 
1972; Kiang et al., 1979; Evans, 1983).

On the basis of a number of single unit studies 
investigating neural response to electrical stimuli, it 
has become obvious that there are a number of fundamental 
differences between the behaviour of electrically and 
acoustically driven auditory nerve fibres. First, the
discharge rate for a single auditory nerve fibre increases over a 20 to 40 dB range for acoustic stimuli, however, the rate increases far more rapidly for electrical stimulation (Kiang and Moxon, 1972; Sachs et al., 1983; Hartmann et al., 1983; Javel et al., in press). This is illustrated in Fig. 2.18. Such a rapid increase in discharge rate results in a small functional dynamic range for electrical stimuli. This limited dynamic range has also been observed psychophysically (Eddington et al., 1978; Tong et al., 1980), indicating the need for considerable signal compression within the coding strategy of a cochlear prosthesis. Second, the width of the peaks in the PSTH are narrower for electrical stimulation than acoustic stimulation, i.e. there is greater synchronization of single unit responses to electrical stimulation therefore giving rise to less stochastic behaviour (Kiang and Moxon, 1972; Sachs et al., 1983; Hartmann et al., 1983; Javel et al., in press). This finding does, however, indicate that at least rudimentary temporal information could be coded by a cochlear prosthesis, and is again supported by psychophysical results (Eddington et al., 1978; Tong et al., 1980). Third, the relatively small latency variations in the responses from fibres of different CF to electrical stimulation is in contrast to the large latency variations inherent in the mechanical transduction process. Finally, electrically stimulated
auditory nerve fibres are not subject to mechanical non-linearities such as two-tone suppression. Such non-linear behaviour, present in normal acoustically driven cochleas, plays an important role in the coding of complex acoustic signals such as speech.

There are therefore, a number of inherent differences in the nature of single unit responses to acoustic and electrical stimuli, although, the effect of any one or more of these differences on the ability of the central nervous system (CNS) to process coded speech is unclear. These differences will cause degradation of the coded signal. However, there are indications that speech can undergo considerable degradation while still being intelligible (Kiang and Moxon, 1972; Evans, 1983). For example, an audiological evaluation of six patients fitted with multi-channel cochlear prostheses, showed that all patients were receiving enough information to significantly improve their speech intelligibility scores (Dowell et al., 1984). A number of patients scored statistically significant speech intelligibility scores when using the cochlear prosthesis without the aid of lipreading.

In order to provide maximum speech intelligibility, a cochlear prosthesis must provide both temporal and discrete place information. The latter can be achieved using a multi-channel electrode array placed
Figure 2.18 Discharge rate in a single auditory nerve fibre as a function of stimulus level for both tone and biphasic electrical current pulse stimulation. The tone was presented at 7.0 kHz (CF) and the current pulses were presented at 200 pps in 100 ms bursts. (adapted from Javel, 1981 and Javel et al., in press).
adjacent to the speech frequency range of the cochlea (Kiang and Moxon, 1972; Kiang et al., 1979; Evans 1983). The speech frequency range (200 - 3500 Hz) is represented within the region from 9 to 26 mm the round window of the human cochlea (Black, 1978). Thus the electrode array would need to be positioned within this region of the cochlea. Furthermore, it would appear from psychoacoustic data that 10 independent channels across the speech frequency range would be necessary as this is the minimum requirement for readily intelligible speech recognition (Flanagan, 1965).

The ability to electrically excite a discrete neural population is therefore, an important requirement for a multi-channel device. A number of investigators have performed single unit studies within the ascending auditory pathway in order to examine the extent of current spread for both monopolar and localized bipolar stimulation regimes (Moxon, 1971; Merzenich and White, 1977; Black, 1978; Black et al., 1983; Hartmann et al., 1983; Javel et al., in press). The results of one study are illustrated in Fig. 2.19, and are typical of results obtained by other investigators.

Recording single unit activity from the central nucleus of the inferior colliculus, Black (1978) was able to plot the degree of current spread for monopolar and bipolar stimulation for a large number of single units representing a spatially wide region of the cochlea.
From the figure it can be seen that monopolar electrical stimulation extends to all regions of the implanted cochlea. The attenuation of the stimulus in the region of the electrode was between 0.6 to 0.8 dB/mm. Such an electrode geometry is not capable of providing discrete place information at sites along the implanted cochlea, although it is capable of providing temporal and intensity cues (Kiang and Moxon, 1972; Merzenich and White, 1977; Black, 1978; Evans, 1983; Hartmann et al., 1983). In contrast, neural excitation from bipolar stimulation is restricted to regions close to the stimulus site.

Attenuation characteristics for bipolar electrodes have been measured in the range 4 to 5 dB/mm (Merzenich and White, 1977; Black, 1978) which is acoustically equivalent (due to the dramatic differences in dynamic range) to several hundred dB/octave, and therefore approaching the steep cut-off slopes of normal cochlear fibre filter functions (Evans, 1983). The greater localization of the excitatory field achieved with bipolar stimulation is significant, and indicates that multi-channel bipolar devices should be capable of providing discrete place information. Indeed, these physiological findings have been supported by psychophysical data from patients implanted with multi-channel scala tympani arrays (Eddington et al., 1978; Tong et al., 1980; Shannon, 1983; Dowell et al., 1984).
**Figure 2.19** The threshold of single inferior colliculus units to electrical stimulation from fine wire electrodes situated 6 to 7 mm from the round window, plotted as a function of the characteristic frequency of the units and their computed distances from the round window. Results due to both monopolar (△) and bipolar (●) stimulation of the scala tympani are shown together with stimulation of the scala tympani relative to an electrode in the scala vestibuli (○). The approximate position of the stimulus site is indicated (■).

(from Black et al., 1983)
The ability to stimulate several discrete auditory nerve populations must be incorporated in an appropriate speech coding strategy. It is generally agreed that there is a need to provide a greater amount of fine temporal coding to improve current speech processing strategies (Evans, 1983). This is one area of major research for groups developing cochlear prostheses.

2.12.2 Evoked potential studies.

(a) Basic observations.

The auditory brainstem response (ABR) consists of a series of far-field potentials evoked by a transient acoustic stimulus such as a click or tone-pip (Jewett et al., 1970; Sohmer and Feinmesser, 1973; Picton et al., 1974). Although the generators of the ABR are known to arise from synchronous activity in auditory centres ranging from the auditory nerve to the inferior colliculus and, for most waves, there exist multiple neural generators (Buchwald, 1983; Britt and Rossi, 1983), these far-field potentials appear relatively simply organized in terms of frequency specificity, amplitude and latency behaviour. Moreover, their responses can be readily interpreted from well known single auditory nerve discharge properties.
Since these well organized, robust potentials can be recorded using non-invasive procedures, the technique has been used extensively in both experimental and clinical investigations. In addition, the technique has been employed in evaluating auditory function in response to electrical stimulation of the cochlea (Merzenich and White, 1977; Miller et al., 1978; Starr and Brackmann, 1979; Simmons, 1979; Dobie and Kimm, 1980; Gyo and Yanagihara, 1980; Yamane et al., 1981; Walsh and Leake-Jones, 1982; Black et al., 1983b; O'Leary et al., 1985).

The nature of the electrically evoked ABR (EABR) will be presented in some detail as the technique has been used extensively in the present research. A typical set of EABR responses is shown in Fig. 2.20 for 0.1 ms duration (50 μs/phase) biphasic current pulses ranging in amplitude from 0.6 - 1.3 mA. For comparison, an ABR series evoked by a 0.1 ms acoustic click over an intensity range of 21 to 86 dB pe SPL, is also included in the figure. The recordings were made in the one cat using the same recording electrode sites (Chapter 4 details the procedure). The ABR was recorded prior to the surgical placement of a banded scala tympani electrode array used to evoke the EABR. During the surgery the cochlea was infused with a solution of neomycin sulphate (Shepherd and Clark, 1984) to ensure that the EABRs were not corrupted by electrophonic activation of intact hair cells (Moxon, 1971).
There are a number of similarities between the electrically and acoustically evoked responses. First, both set of responses consist of four waves (although wave I has been somewhat obscured in the EABR because of the need to suppress the electrical artefact). Moreover, the response waveforms show similar morphology. Second, the amplitude of both responses increases, and wave latencies decrease with stimulus intensity. Third, although absolute latencies for the ABR series are somewhat longer than their EABR counterpart, the interpeak latencies are approximately the same (about 0.8 - 1.0 ms). Other investigators have formed similar conclusions following comparison of ABRs and EABRs in a variety of experimental animals (Miller et al., 1978; Gyo and Yanagihara, 1980; Yamane et al., 1981; Black et al., 1983). In addition, similar observations have been made following the recording of EABRs in human cochlear prosthesis patients (Starr and Brackmann, 1979).

From the present observations, together with previous single unit studies investigating electrical stimulation of the cochlea, it is reasonable to conclude that ABRs and EABRs are derived from common neural generators.

The significantly longer latency of the ABR in comparison with the EABR (approximately 1.0 - 1.5 ms) reflects the differing physiological mechanisms involved in the generation of action potentials within auditory
Figure 2.20  (a) EABRs evoked by a 0.1 ms biphasic current pulse stimulated via a bipolar scala tympani electrode. The electrical artefact has been suppressed.

(b) ABRs evoked by a 0.1 ms click. Stimulus intensity in dB pe SPL re 20 uPa. Wave III is shown in both examples (arrow). The amplitude calibration line is 1.0 uV.
Stimulus: 50 µs/phase current pulse

Stimulus: 0.1 ms acoustic click
nerve fibres. While the electrically evoked response directly excites the auditory nerve and is subject to minimal delays (due to the capacitance of the neural membrane), the acoustically evoked response is subject to several delays, including the acoustic travel time between the transducer and the inner ear, the mechanical delay associated with basilar membrane motion and the synaptic delay experienced at the hair cell auditory nerve junction.

(b) Input-output and latency functions.

In order to examine some of the important physiological differences between the electrically and acoustically evoked brainstem responses, it is convenient to represent the waveforms as input-output and latency functions. Figure 2.21 illustrates two auditory brainstem response input-output functions, one evoked acoustically and the second, electrically. These graphs were derived by plotting the amplitude of the evoked response (in this example, wave III in Fig. 2.20) against stimulus intensity. To compare the responses, the stimulus current has been expressed in dB. These results highlight one important difference between the two forms of stimuli, namely the small functional dynamic range of the electrically evoked response. These findings are consistent with the single unit and psychophysical results described previously.
Latency functions for both electrically and acoustically evoked responses are illustrated in Fig. 2.22. In this example Wave III latency (from the responses illustrated in Fig. 2.20) are plotted against stimulus intensity. The latency of the response, in both cases, is reduced with increasing stimulus intensity. However, there are three significant differences between the two forms of evoked response. First, the EABR has a significantly shorter latency than the ABR. The mechanisms underlying the latency differences have been discussed previously. Second, the narrow dynamic range associated with the electrically evoked responses is in contrast with the response evoked acoustically (as already observed in the input-output data). Third, the narrow shift in latency with stimulus intensity observed electrically, is in contrast with the relatively large shifts observed acoustically. Again, these findings highlight important physiological differences between the acoustically stimulated and electrically stimulated cochlea.

Acoustic stimulation, using short duration transient stimuli such as an acoustic click, excites a wide region of the cochlear partition (Teas et al., 1962; Eggermont and Don, 1980; Evans and Elberling, 1982; Shepherd and Clark, 1985). As the stimulus intensity is increased, there is a greater contribution to the evoked potential from the more basal fibres within the cochlea.

2-61
Figure 2.21 Electrically and acoustically evoked brainstem response input-output functions. These data were derived from the responses illustrated in Fig. 2.20.
Figure 2.22 Latency functions for acoustically and electrically evoked brainstem responses. These data were derived from the responses illustrated in Fig. 2.20.
This increase in contribution from basal fibres reduces the mechanical travelling time along the cochlear partition at which the maximum excitation occurs, therefore reducing the latency of the evoked response. The narrow latency shift experienced with electrical stimulation can be reasonably explained in terms of synchrony. At stimulus levels just above threshold the degree of synchrony is low, the evoked potential waveform is relatively broad and the latency is relatively long. Within a few dB increase in current, however, the response becomes highly synchronized and the latency reduced. Similar observations have been made from single unit studies (van den Honert and Stypulkowski, 1984; Javel et al., in press).

These observations emphasize important differences between direct electrical stimulation of the auditory nerve and the spatio-temporal dependent processes that dominate acoustic stimulation.

(c) The relationship between EABR and electrically evoked N1 recordings.

The relationship between the behaviour of the EABR and the electrically evoked compound action potential (N1E) recorded at the VIII nerve is illustrated in the input-output and latency functions of Figs. 2.23
and 2.24 respectively. The recordings were made in the one cat, using the same bipolar scala tympani stimulating electrode and electrical stimulus. The EABR was recorded using a standard recording electrode configuration and 100 stimuli were averaged for each recording. Wave IV was used to derive the input-output and latency data. The N1E was recorded by placing a gross silver ball electrode on the exposed VIIIth nerve just medial to the internal auditory meatus. Ten stimuli were averaged for each N1E recording. The short latency of the N1E (Fig. 2.24) indicates that the recording was made from the auditory nerve and not postsynaptic regions of the cochlear nucleus.

Although wave IV of the EABR has a considerably smaller amplitude (Fig. 2.23) and longer latency (Fig. 2.24) than the N1E, both intensity functions illustrate how closely EABR wave IV reflects the behaviour of the N1E. Similar findings have been reported for ABRs and the acoustically evoked N1 (Huang and Buchwald, 1978). These results suggest that there is little additional modification of the N1E amplitude and latency responses occurring within the auditory brainstem beyond the level of the auditory nerve. It is, therefore, reasonable to interpret changes in the EABR as changes originating within the auditory periphery.

2-65
Figure 2.23 Electrically evoked N1 and EABR input-output functions obtained from the same experimental preparation. Biphasic current pulses stimulated the auditory nerve via a bipolar scala tympani electrode.
Figure 2.24  EABR and electrically evoked N1 latency functions.
(d) Interpreting neural excitation patterns from EABR input-output functions.

Using the binaural properties of single units within the central nucleus of the inferior colliculus to investigate the degree of spatial spread of stimulus currents for a variety of electrode geometries, Merzenich and White (1977) correlated the spread of electrically evoked neural excitation with the amplitude of the brainstem response and concluded that the amplitude of the EABR is monotonically related to the number of neurones being excited. This relationship has also been suggested by others (Miller et al., 1978). On the basis of these observations it is reasonable to interpret the growth of amplitude of the EABR with stimulus current (i.e. the input-output function) in terms of spread of neural excitation within the electrically stimulated cochlea.

An example of the use of the EABR to highlight differences in neural excitation patterns is illustrated in Fig. 2.25. In this figure EABR input-output functions are shown for three bipolar scala tympani electrode configurations; narrow (0.45 mm), medium (1.20 mm) and wide (1.95 mm) inter-electrode spacings. The same recording electrodes and electrical stimuli were used to obtain the three set of data. As the figure illustrates, increasing the inter-electrode spacing of a bipolar electrode significantly increases the spread of
neural excitation as indexed by the slope of the input-output functions. This is particularly evident for mid-dynamic range currents (0.5 - 1.8 mA), although threshold for the wide spaced pair (1.95 mm) was also lower than the threshold for the narrower spaced electrodes. Similar observations have been made in cochlear implant patients implanted with the same type of electrode array. By increasing the inter-electrode spacing patients' threshold and comfortable loudness current levels are reduced (R.C. Dowell, personal communication). In addition, Pfingst et al. (1979), have reported reductions in perceptual thresholds when inter-electrode spacings were increased in monkeys implanted with scala tympani electrodes.

Another point of interest present in Fig. 2.25, is the fact that the maximum EABR amplitude evoked by the 1.20 mm spaced electrode pair was similar to that evoked by the wider spaced electrodes, and suggests that at high stimulus currents (1.8 - 2.0 mA) both electrode geometries were exciting a common neural population. Moreover, the asymptotic nature of the input-output function produced by the 1.95 mm spaced electrodes, evident at moderate and high stimulus currents (1.2 - 2.0 mA), suggests that at these stimulus levels, an increase in stimulus current would evoked little additional neural activity.
Figure 2.25 Comparison of ABR input-output functions for three different bipolar scala tympani electrode configurations; narrow (0.45 mm), medium (1.20 mm) and wide (1.95 mm) inter-electrode spacings.
Figure 2.26 illustrates a second example of the use of the EABR input-output function in interpreting neural excitation patterns. In this example, input-output functions are shown for charge balanced biphasic current pulses ranging in pulse width from 50 - 400 us per phase. For moderate suprathreshold stimulus levels it is clear that the amplitude of the brainstem response is charge dependent, i.e. the EABR amplitude depends both on stimulus current and pulse width. Moreover, these data suggest that this charge dependent relationship, although monotonic, is not linear. These observations are consistent with recent psychophysical data obtained from implant patients (Shannon, 1985).

At threshold and near threshold stimulus currents in this example, the charge dependent relationship is not evident. This is in conflict with both previous single unit (van den Honert and Stypulkowski, 1984; Javel et al., in press; Parkins, in press) and psychophysical (Shannon, 1985) observations. The probable explanation of the observed difference between present and previous data at threshold currents may be due to the fact that the animal used in the present example had normal hearing. It is probable that at low stimulus currents (50 - 200 uA) the responses were dominated by electrophonic activation of intact hair cells, a process that is independent of pulse duration (Black et al., 1983b).
Figure 2.26 Comparison of EABR input-output functions for six different pulse widths (50 - 400 us/phase). A monotonic relationship between pulse width (and charge per phase) and the amplitude of the EABR is evident.
The apparent monotonic relationship between amplitude of the electrically evoked brainstem response and the extent of neural excitation, is consistent with previous physiological, psychophysical and clinical results, and therefore has potentially important applications both experimentally and clinically.
3.1 Introduction.

The success of a cochlear prosthesis depends, in part, on the long-term survival of the residual primary auditory neurones. The success of these devices may be compromised by a number of factors including: (i) an inadequate spiral ganglion cell population prior to the implantation of the prosthesis due to adverse cochlear pathology; (ii) trauma associated with the insertion of the scala tympani electrode array; (iii) infection following implantation; (iv) adverse foreign body reaction in response to the presence of the electrode array; (v) the effect of electrical stimulation per se on the cochlea and its neural elements. It is the purpose of this chapter to review these histopathological factors; the concluding section of this chapter will present an outline of the research performed in the present thesis.
Degeneration of the Auditory Nerve.

The sequence of first-order neural degeneration following cochlear pathology has been well studied in both human and experimental animals. Spiral ganglion cells are bipolar, their axons project centrally to the cochlear nucleus and their dendrites project radially towards the organ of Corti. When an axon or dendrite is injured two degenerative processes are observed. Wallerian degeneration occurs within a few days of the injury and results in a complete breakdown of the distal portion of the axon which has been separated from its perikaryon. Anterograde degeneration, effecting the proximal portion of the axon in the direction of the ganglion cell body, is observed over a longer time period. This form of neural degeneration is peculiar to bipolar sensory neurones where it normally extends over one or two internodal lengths, occurring slowly and after some time being replaced by axonal regeneration. However, approximately 90 to 95% of neurones of the spiral ganglion experience anterograde degeneration to include the cell body, therefore excluding the possibility of regeneration (Kerr and Schuknecht, 1968; Spoendlin, 1972; Johnsson, 1974; Spoendlin, 1975; Spoendlin and Suter, 1976). Some sensory neurones are more sensitive to anterograde degeneration than others; for example, in comparison to the extensive degeneration observed with first-order auditory neurones, most
neurones within the vestibular nerve resist extensive anterograde degeneration following axonal or dendritic injury (Spoendlin and Suter, 1976). Similar observations have been made in relation to neural degeneration as a result of presbycusis (Iurato, 1967; p 253).

The extent and rate of neural loss appears to depend on a number of factors including the nature and extent of the cochlear trauma and the species under investigation. For example, surgical insult of the organ of Corti in guinea pig results in extensive localized degeneration within three weeks of the damage (Webster and Webster, 1978), whereas direct administration of an ototoxic drug in the same species requires a period of sixty days for the degenerative process to come to completion (Terayama et al., 1979). There is also evidence of greater spiral ganglion cell survival in human cochleas than would be predicted from experimental animal data with similar pathologies (Ylikoski et al., 1981; Hinojosa and Marion, 1983).

Degeneration of the spiral ganglion may be initiated by direct trauma to the dendrite when, for example, the osseous spiral lamina is fractured. However, in the majority of pathologies, degeneration is preceded by atrophy of the organ of Corti. It is still not clear what structure within the organ of Corti is associated with the initiation of neural degeneration; a
number of investigators cite the loss of the inner hair cell as the precursor to anterograde degeneration (Bredburg, 1968; Spoendlin, 1972; Spoendlin and Suter, 1976), while a second group maintain that the collapse of the supporting structure within the organ of Corti initiates degeneration (Kerr and Schuknecht, 1968; Johnsson, 1974; Schuknecht, 1974, Otte et al., 1978). All investigators agree, however, that the change that results in anterograde degeneration initially damages the peripheral fibre innervating the inner hair cell.

Damage restricted to the outer hair cells of the organ of Corti results in minimal degeneration of the spiral ganglion (Spoendlin, 1975). This observation is consistent with the present knowledge of the innervation patterns of the sensory hair cells (Section 2.5).

The sequence of anterograde degeneration of first-order auditory neurones has been well documented (Powell and Erulkar, 1962; Schuknecht, 1973; Spoendlin and Suter, 1976; Arnesen et al., 1978; Terayama et al., 1979). Initially, there is an accumulation of granular organelles and cytoplasmic vesicles. This initial activity within the cytoplasm may reflect an increase in protein synthesis within the injured cell. Presumably, digestive enzymes would be required to assist in the removal of products associated with cellular injury. Ribosome, granular endoplasmic reticulum and lysosome activity would be expected to increase. This initial
reaction is followed by a swelling of the myelinated nerve fibres and a shrinkage of the axoplasm. Within several days the dendrites disappear, the myelin lamellae become disrupted and macrophages begin to infiltrate the nerve fibre initiating a long phagocytotic process. Schwann cells also undergo degeneration and although the spiral ganglion cell body is present, it displays an irregular and eccentric nucleus. After the first week following the trauma, the degenerative process appears to slow down. Indeed, at this stage regenerating dendritic sprouts are often observed within the modiolus. Finally, however, the degenerative process prevails and the active neural sprouting gives way to a dramatic loss of dendrites and their cell bodies by approximately the start of the second month. By the end of this month only 5 to 10% of the spiral ganglion population survive. By the end of the third month the degenerative process has stabilized (Spoendlin, 1975; Spoendlin and Suter, 1976).

The surviving ganglion cells are not normal in appearance, their dendrites are missing and their nuclei are pyknotic and displaced towards the axonal side of the cell (Kerr and Schuknecht, 1968; Schuknecht, 1974). Frequently, a large vacuole is observed at the dendritic pole of the cell. The remaining ganglion cells consist of two populations; the small, unmyelinated Type II cell and a number of Type I cells without their myelin (Type 3-5
Although significant neural elements can survive anterograde degeneration following damage to the organ of Corti, it should be noted that more severe pathologies such as bacterial labyrinthitis and vascular occlusion, may result in more extensive spiral ganglion cell loss than experienced with pathologies initially affecting Corti's organ (Spoendlin, 1975). Presumably the degenerative mechanisms involved with these pathologies differes somewhat from the anterograde degeneration that occurs following damage to the peripheral dendrite.

3.3 Transneural Changes Following Degeneration of the Auditory Nerve.

The survival of neurones within the more central nuclei of the auditory system following peripheral cochlear damage, is an important consideration for groups developing cochlear prostheses. Certainly, spiral ganglion cell loss can be correlated with the degree of degeneration of primary axonal endings within the ipsilateral ventral cochlear nucleus (Miller et al., 1980). In addition to these findings, a number of investigators have examined the extent of transneuronal changes within higher order auditory nuclei following cochlear damage. A loss of first-order auditory neurones will result in a decrease in the size of neurones from the ipsilateral ventral cochlear nucleus,
the superior olivary nucleus, the contralateral medial trapezoid, the lateral lemniscal nuclei (Powell and Erulkar, 1962), and the central nucleus of the inferior colliculus (Webster and Webster, 1978; Miller et al., 1980). These cellular changes have been observed over the first 60 days following cochlear damage, after which cell morphology appears to stabilize (Powell and Erulkar, 1962; Hall, 1975). Similar transneuronal cell changes have been reported following neonatal sound deprivation (Coleman, 1981; Webster, 1983). Reduction in neuronal soma sizes were reported in the large spherical cells of the anterior ventral and dorsal cochlear nuclei (Coleman, 1981), the globular cells of the ventral cochlear nucleus, the principal cells of the medial nucleus of the trapezoid body and the spindle shaped principal cells of the inferior colliculus (Webster, 1983).

Although reports examining changes in post-first-order auditory neurones following partial or complete degeneration of the auditory nerve have shown no evidence of cell loss within these auditory nuclei, there is evidence of the presence of Fink-Heimer positive degenerative debris within these auditory centres during the period that the morphological changes were observed (Morest et al., 1979).

An understanding of the effects of electrical stimulation of non-active neurones in chronically deafened animals is necessary. Such stimulation may be
physiologically beneficial as there is evidence that intracochlear electrical stimulation is capable of restoring normal levels of cytochrome oxidase in higher order auditory nuclei of chronically deafened animals (Merzenich et al., 1979). The histochemical assessment of cytochrome oxidase provides an indication of neuronal energy utilization, and has been shown to be significantly higher in chronically deafened cochleas subjected to periods of electrical stimulation, compared with deafened, non-stimulated controls.

3.4 Auditory Nerve Survival in Profound Sensorineural Deafness.

A number of reports have examined the residual spiral ganglion cell populations from patients suffering from profound or total hearing loss (Kerr and Schuknecht, 1968; Otte et al., 1978; Hinojosa and Lindsay, 1980; Hinojosa and Marion, 1983; Hinojosa et al., in press). A summary of results from two of these studies is presented in Table 3.1. Although there is considerable variation in results between the two studies, both illustrate the significant ganglion cell populations present in patients that have been profoundly or totally deaf for many years. Indeed, only three of 16 cochleas examined by Hinojosa and Marion (1983) had ganglion cell populations of less than 10,000 - a number considered as a minimum requirement for speech comprehension (Otte et al., 1978).
Summary of results from two studies in which spiral ganglion cell populations were correlated with cochlear pathology. All cochleas from both studies were from profound or totally deaf patients.†

<table>
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<tr>
<th>Diagnosis</th>
<th>Total</th>
<th>Over 10,000 Cells</th>
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<tbody>
<tr>
<td>Viral labyrinthitis and sudden deafness</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Bacterial labyrinthitis</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Cochlear dysplasia</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Congenital syphilis</td>
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<td>1</td>
</tr>
<tr>
<td>Otosclerosis</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Deafness from drugs</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Transverse fracture</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Vascular occlusion</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Presbycusis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ménière's disease</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>28 (45%)</td>
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<table>
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<th>Age (yr) &amp; Sex</th>
<th>Diagnosis</th>
<th>Ganglion Cell Count</th>
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<td>Viral meningogenic labyrinthitis</td>
<td>7,305</td>
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<tr>
<td>2 6, M</td>
<td>Viral endolymphatic labyrinthitis</td>
<td>19,826</td>
</tr>
<tr>
<td>3 46, F</td>
<td>Bacterial labyrinthitis</td>
<td>Zero</td>
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<tr>
<td>4 87, M</td>
<td>Bacterial labyrinthitis</td>
<td>22,125</td>
</tr>
<tr>
<td>5 80, F</td>
<td>Head trauma</td>
<td>15,110</td>
</tr>
<tr>
<td>6 60, F</td>
<td>Otosclerosis</td>
<td>16,127</td>
</tr>
<tr>
<td>7 68, F</td>
<td>Otosclerosis</td>
<td>13,139</td>
</tr>
<tr>
<td>8 67, M</td>
<td>Streptomycin ototoxicity</td>
<td>19,608</td>
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<td>9 79, M</td>
<td>Paget's disease</td>
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<td>10* 77, M</td>
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</tr>
<tr>
<td>16 53, F</td>
<td>Congenital aplasia (organ of Corti)</td>
<td>16,663</td>
</tr>
</tbody>
</table>

† a) data from Otte et al., 1978.

b) data from Hinojosa and Marion, 1983.

Normal human spiral ganglion population is approximately 30,000.
There are few consistent pathological findings arising from the histological evaluation of cochleas from profoundly and totally deaf patients. Certainly, there is severe and often total destruction of the organ of Corti. However, there is often a significant spiral ganglion cell population associated with such a loss—a finding that is in conflict with the degeneration pattern observed in experimental animals following the loss of the organ of Corti (Schuknecht, 1974; Spoendlin, 1975). Although there is some evidence indicating a correlation between cochlear pathology and the residual spiral ganglion cell population, there are very large variabilities in ganglion cell survival rates, even among cochleas with a common pathology. Presumably, this is due to the extent and severity of the pathology, the time period between the pathology and histological evaluation, and the effect of presbycusis in older patients.

Such variability in surviving spiral ganglion cell numbers is well illustrated by examining the cell counts for patients 3 and 4 of Hinojosa and Marion's study (Table 3.1b). Both patients were diagnosed as having bacterial labyrinthitis, however one patient had in excess of 20,000 ganglion cells while the second patient had none. This extreme example highlights the need for a technique capable of quantitatively assessing ganglion cell populations among perspective implant patients. Currently no satisfactory technique exists.
From the examination of the data currently available, it would appear that ganglion cell populations would generally be largest for patients suffering from sudden deafness, Menier's disease, presbycusis and ototoxic drugs; somewhat less for vascular occlusions, temporal bone fractures, cochlear dysplasia and otosclerosis; and least for viral and bacterial labyrinthitis. In addition, temporal bone fractures, viral and bacterial labyrinthitis, otosclerosis and cochlear dysplasia show the greatest variation in ganglion cell survival among patients with a common pathology.

Although it is not known what fraction of the total neuronal population is required to be intact to allow useful hearing from electrical stimulation of the cochlea, recent conditioning experiments in deafened cats have shown no difference in amplitude and frequency difference limens for intracochlear electrical stimulation in animals with residual spiral ganglion cell populations ranging from 8-44% of normal (Black et al., 1983c). These results suggest that even patients with a severe spiral ganglion cell loss should receive important intensity and stimulus rate cues from a cochlear implant.
3.5 **Electrode Insertion Trauma: Histopathological Considerations.**

Investigators have used a number of anatomical approaches to electrically stimulate the auditory nerve. These include direct electrical stimulation of the auditory nerve using a wire bundle inserted into the nerve via a hole drilled into the modiolus (Simmons, 1966), the insertion of platinum/iridium (Pt/Ir) wire electrodes into the scala tympani via fenestrations made into the otic capsule following "compartmentalizing" of the scala with Silastic® (Chouard et al., 1983), and the placement of Pt/Ir ball electrodes into holes created in the otic capsule without invading the scala (Banfai et al., 1980). However, the most frequent approach has been to introduce the array along the scala tympani via the round window (Michelson, 1971; Clark et al., 1979; Hochmair-Desoyer and Hochmair, 1980; White et al., 1983; Clark et al., 1984).

It is essential that the surgical placement of the electrode array does not result in trauma that could reduce the residual spiral ganglion cell population. The degree of insertion trauma is likely to vary from one type of array to another depending on the mechanical and physical properties of the electrode, the surgical technique used to place it, and its implantation site.
In a recent study (Shepherd et al., 1985) the banded scala tympani array developed at the University of Melbourne in association with Nucleus Limited, was inserted into nine human cadaver temporal bones using the surgical protocol developed for the insertion of this array (Clark et al., 1984). The electrode array consisted of 22 Pt band electrodes on a Silastic® MDX-4-4210 carrier (Clark et al., 1983), designed for insertion to 25 mm along the scala tympani (Fig. 3.1). Histological examination of these human cochleas revealed four distinct sites of trauma: 1) spiral ligament within the scala tympani; 2) osseous spiral lamina; 3) basilar membrane; and 4) Reissner's membrane. The results of this study are summarized in Fig. 3.2, where the extent and frequency of the four modes of damage are plotted against distance along the cochlea spiral.

Tears along the spiral ligament of the scala tympani were the most common forms of trauma experienced in the study. Generally occurring in a 7 - 13 mm zone from the round window, these tears were probably a result of the electrode coming into contact with, and being forced along, the outer wall of the scala tympani. This form of damage would be expected to evoke a soft tissue reaction (Johnsson et al., 1982). However, it is possible that osteogenisis would also occur as new bone has been reported in animal studies following damage to the endosteum (Schindler and Merzenich, 1974).
Figure 3.1 Diagram of the banded scala tympani electrode array. All dimensions are in millimeters. (from Shepherd et al., 1984).

Figure 3.2 Summary of electrode insertion trauma for nine cochleas. Mode of trauma is plotted as the number of cochleas versus distance from the round window (0 mm). (from Shepherd et al., 1984).
Provided that damage was restricted to the spiral ligament this type of trauma would not be expected to result in neural degeneration (Clark et al., in press).

The localized damage to both the basilar membrane and the osseous spiral lamina would, however, be expected to result in severe neural degeneration localized to the trauma (Simmons 1967; Clark, 1977; Schindler et al., 1977; Sutton et al., 1980). Such trauma highlights the care that is required during the insertion of a scala tympani electrode array. The fracture of the osseous spiral lamina was a result of the electrode buckling in the basal turn, which also resulted in localized damage to the basilar membrane, while the small perforation of the basilar membrane 15 mm from the round window occurred as a result of the surgeon forcing the electrode past the point of first resistance. It should be emphasized that damage to the basilar membrane and the osseous spiral lamina occurred in only two of nine cochleas, and was localized to very small regions of each cochlea (Fig. 3.2).

Tears of Reissner's membrane were observed in both implanted and control (non-implanted) cochleas. Although, in a few cases, damage to Reissner's membrane could have occurred when the spiral ligament was damaged, the majority of tears were probably due to preparation artefact as there was no statistical difference between the length and number of tears in the control cochleas.
when compared with the implanted cochleas (Clark et al., in press). On the limited data available from animal studies it would appear that localized tears of Reissner's membrane would not contribute to the degeneration of the auditory nerve providing the membrane is allowed to heal (Duvall and Rhodes, 1967; Clark, 1977).

These results indicate that the insertion of the banded scala tympani array produces minimal damage that would result in subsequent neural degeneration, providing that the array is not forced past the point of first resistance. Similar studies using surface preparation techniques have confirmed these histological findings (Clifford and Gibson, in press; Franz and Clark, in press), and refinements to the surgical technique have been recommended to further reduce the chances of insertion induced trauma (Franz and Clark, in press).

3.6 Foreign Body Reactions.

The implantation of a scala tympani electrode array may evoke a foreign body reaction. Such an inflammatory response will proceed from an acute to a chronic reaction and finally, providing the stimuli evoking the response subside, to a resolved response characterized by the encapsulation of the electrode array with mature fibrous tissue.
A foreign body reaction is a very dynamic physiopathological reaction, with many stimuli acting simultaneously to maintain the inflammatory process. Such a reaction is therefore quite variable among individuals of the same species (Homsy, 1970). There are a number of stimuli capable of prolonging the active inflammatory process. Mechanical irritation of surrounding tissue due to the shape and rigidity of the implant are of particular concern when implanting prostheses within tissue subjected to extensive movement such as muscle (Wood et al., 1970; Coleman et al., 1974). Many implantable materials are not truly inert, monomer or catalyst residues within the polymer may gradually leach out onto the tissue resulting in necrosis. Other chemical irritants such as metal ions may be released as a result of the dissolution of metal within the corrosive environment of the body (Coleman et al., 1974; Loeb, 1983). Indeed, the majority of metals corrode when implanted in tissue (McPadden, 1963; Brown and Merrit, 1980; Clark and Williams, 1983). This is a particular concern in electrically active prosthetic devices where the charge injection process places additional electrochemical stress on the metal electrodes.

The presence of a foreign body predisposes to infection, which is capable of considerably extending the active inflammatory process, and if left unchecked could
result in widespread neural degeneration within the cochlea (Simmons, 1967; Clark, 1977; Cranswick et al., in press).

In addition to these stimuli, there also exists a number of possible inflammatory stimuli peculiar to electrically active implants. First, an adverse electrical stimulation regime will evoke an inflammation reaction (Pudenz et al., 1977). Second, electrochemical effects of prolonged electrical stimulation resulting in metal dissolution (Agnew et al., 1977; McHardy et al., 1980), would also contribute to an inflammatory reaction. Third, the increased activity of excitable tissue in response to electrical stimulation, and the possible effects of the stimulation on blood supply due to arterial sphincter contraction, may result in metabolic supply and removal difficulties at the stimulus site (Loeb, 1983). Changes in local homeostasis such as these may also contribute to an inflammation reaction.

The histological evaluation of an implanted foreign body is an important means of assessing the biocompatibility of an implant material. Cellular injury, either of traumatic or biochemical origin, stimulates fibroblast activity so as to encapsulate the offending foreign body. It would appear that the thickness of the fibrous tissue response is a gross measure of the degree of biocompatibility (Homsy, 1970). Furthermore, in order to ensure an adequate
evaluation of mechanically induced inflammation, it is necessary to implant the foreign body in the site intended for its use, taking care to use an appropriate animal model.

The histopathological assessment of chronic, unstimulated intracochlear electrodes is now well documented (Simmons, 1967; Clark, 1973; Clark et al., 1975; Schindler and Merzenich, 1974; Schindler et al., 1977; Sutton et al., 1980; Shepherd et al., 1984). These long-term experimental studies have used a variety of electrode designs, materials, and surgical techniques, however, all studies have shown that chronic intracochlear implantation is well tolerated by the cochlea and the peripheral auditory nerve fibres adjacent to the implant, provided the insertion procedure was free of trauma and infection, and the electrode was manufactured from appropriate biomaterials using established clean room techniques. These studies indicate that the scala tympani is a suitable site for chronic electrode implantation.

3.7 Electrical Stimulation of Neural Tissue: Histopathological Considerations.

The major remaining histopathological consideration in assessing the efficacy of a cochlear prosthesis, is the effect of electrical stimulation on the primary auditory nerve fibres.
The development of biologically safe means of electrical stimulation of neural tissue is currently receiving considerable attention. However, the mechanism by which an electrical stimulus — applied through a noble metal electrode — results in neural damage is poorly understood. Direct and radiofrequency (r.f.) currents are known to result in the destruction of neural tissue due to electrolysis and thermal injury respectively (Lilly, 1961). Non-destructive electrical stimulation can be achieved by the use of charge balanced biphasic pulsatile stimuli (Lilly, 1961; Mortimer et al., 1970). Although maximum biologically safe stimulus regimes have yet to be clearly defined, the evidence from a number of investigators suggests that charge density per phase (uC.cm\(^{-2}\) geom. per phase) and total charge injection (uC) are important parameters when establishing biologically safe stimulating levels (Yuen et al., 1981; Agnew et al., 1983). Charge injected per phase (uC per phase) has also been considered an index of neural degeneration (Pudenz et al., 1975; Pudenz et al., 1977; Brown et al., 1977).

A maximum electrochemically safe stimulation regime for Pt electrodes has been defined (Brummer et al., 1977). This stimulation regime consists of short duration charge balanced biphasic pulses at a maximum charge density of 300 uC.cm\(^{-2}\) geom. per phase.
Stimulating within this limit ensures that all charge injection is achieved by reversible electrochemical reactions, therefore ensuring that no new electrochemical products are introduced into the biological environment. It does not follow, however, that this limit is biologically safe.

There have been a limited number of studies examining the histopathological effects of chronic modiolar and intracochlear electrical stimulation. Simmons (1979) reported little neural degeneration following short periods of direct auditory nerve stimulation in five cats for periods of up to 40 hours during an implantation period of 16 months. A biphasic monopolar stimulation regime was used, delivering a charge density of 230 uC.cm\(^{-2}\) geom. per phase. Walsh and Leake-Jones (1982) reported stimulus induced changes in the EABR occurring at charge densities of 100 to 200 uC.cm\(^{-2}\) geom. per phase for bipolar intracochlear stimulation using a charge balanced biphasic stimulus. Significantly, these investigators reported that charge densities in the order of 20 to 40 uC.cm\(^{-2}\) geom. per phase did not produce changes in the EABR although stimulation periods of up to 800 hours were used. Duckert and Miller (1982), stimulated guinea pig cochleas using various levels of sinusoidal current for a period of three hours. At charge densities within the electrochemically safe limits, the cochleas showed little
gross morphological change. For stimulation parameters in excess of these safety limits, however, loss of hair cells, atrophy of the organ of Corti and lesions in the basal turn overlying the electrode, were recorded. The authors considered this damage to be due to gas evolution from the stimulating electrodes. Such stimulus levels are significantly greater than those used in clinical applications.

A number of investigators have evaluated the neuropathological effects of electrical stimulation at sites other than the auditory nerve. In a series of well controlled experiments, Pt disc electrodes were placed on the cortex of cats and stimulated for 36 hours at various charge densities in order to establish a neural damage threshold (Pudenz et al., 1975; Agnew et al., 1975). At a charge density of 38 uC.cm\(^{-2}\) geom per phase no neural damage was observed at either the light or electron microscope levels, however, at charge densities of 49 uC.cm\(^{-2}\) geom. per phase and above, progressively greater neural damage was observed (Pudenz et al., 1977). These authors considered that current flow rather than electrochemical reactions were more important in causing the neural damage observed (Pudenz et al., 1975 b). Stimulus induced damage varied with charge density (Agnew et al., 1975). At threshold levels of injury vacuoles within the cellular cytoplasm together with limited intracellular derangement were
noted. At moderate charge densities, myelin lamellar disarray together with mitochondrial swelling was reported. Cellular debris and macrophages were also observed. Severe neural damage was indicated by the loss of vesicles at synaptic junctions, markedly ballooned mitochondria, severe cytoplasmic vacuolation and membrane disruption. The authors considered this damage to be irreversible (Agnew et al., 1975).

In a more chronic study Brown and his associates (Brown et al., 1977), stimulated the surface of monkey cerebellum using a capacitively coupled monophasic pulse for a 205 hour period. No neural damage was found when a charge density of 7.35 uC.cm\(^{-2}\) geom. per phase was used, however, at all higher charge densities tested (35.5, 71.0, 148.0, and 326.0 uC.cm\(^{-2}\) geom. per phase) neural damage was reported and found to be directly related to the charge density delivered. It should be noted that these investigators used a stimulus regime having a pulse width of 1.0 ms; considerably wider than the pulses generally used for excitation of neural tissue, and possibly resulting in electrolytic injury (Lilly, 1961). Dissolved Pt would probably result in neural damage as Pt salts are known to be toxic to neural tissue (Agnew et al., 1977).

The significance of charge density as the most important stimulus parameter correlating with neural damage was recently demonstrated by Yuen and his
colleagues (Yuen et al., 1981). Using Pt electrodes these investigators applied a charge balanced biphasic stimulus to the cortex of experimental animals for stimulus periods of 1.5 to 50 hours at charge densities of 40 to 400 uC.cm\(^{-2}\) geom. per phase. Slight neural damage was reported at 40 uC.cm\(^{-2}\) geom. per phase. Damage increased with charge density to a maximum of 100 uC.cm\(^{-2}\) geom. per phase, above which the degree of damage plateaued. These authors considered that the total charge injected was a secondary stimulus parameter related to stimulus induced neural damage.

The effect of total charge injection on neural degeneration has also been demonstrated by Agnew et al. (1983). In an acute study, these investigators stimulated the cortex of cats for a period of four hours using a charge balanced biphasic stimulus, charge density and total charge injected were varied to examine the effect of each parameter on neural degeneration. The study showed no histological damage, when compared with a control, for a charge density of 20 uC.cm\(^{-2}\) geom. per phase at 50 pulses per second (pps). Slight, local neural degeneration was reported at 100 uC.cm\(^{-2}\) geom. per phase at 20 pps, with more extensive neural damage associated with a stimulus of 100 uC.cm\(^{-2}\) geom. per phase at 50 pps.
Although there is some variability in the stimulus threshold at which neural damage has occurred, these studies indicate that the maximum biologically safe stimulus regime is considerably less than the electrochemically safe maximum defined by Brummer and his associates (Brummer et al., 1977). Significantly, these studies have consistently shown a correlation between charge density and the degree of neural degeneration. Such findings have important implications for electrode designs, in particular, the need to maintain electrode areas as large as possible.

The major limitation of these contributions has been the relatively short stimulation times employed. There is no doubt that more long-term studies are required. Moreover, variations in electrode geometry, stimulation site and, perhaps most importantly, stimulus regime, make it imperative that experimental studies - using the electrode, stimulus parameters and stimulation site of the prosthesis under investigation - are performed prior to the clinical application of the device.

3.8 Outline of Thesis Research.

It is apparent, from the preceding discussion, that there is now a significant body of evidence to suggest that primary auditory neurones can survive electrode insertion and chronic implantation. There is,
however, limited data examining the pathological and physiological effects of long-term electrical stimulation on these neurones. The present study includes an investigation into the histopathological and physiological effects of chronic intracochlear electrical stimulation upon the cochlea in general, and the spiral ganglion in particular (Chapter 4). In order to closely model the University of Melbourne/Nucleus Limited Cochlear prosthesis, electrodes in this study were manufactured using the same materials and techniques as those used in the manufacture of the Cochlear prosthesis. Moreover, the dimensions used in this study were based on the human array so as to ensure the production of similar charge densities. Animals in the present study were stimulated continuously using a stimulus regime operating in the upper range and identical with that used in the Cochlear prosthesis.

The electrical stability of the stimulating electrodes were examined by monitoring their impedance periodically. As a control, the impedance of identical electrodes stimulated in normal saline were also measured (Chapter 5). Knowledge of the range of electrode impedances experienced following chronic electrode implantation and electrical stimulation will have important implications for the design of the neural stimulator.
Implantable devices are subject to a severe corrosive environment. Neural stimulators are subjected to additional corrosive stresses as a result of the charge injection process (Lilly, 1961; Brummer et al., 1983). Dissolution of metal electrodes is highly undesirable, as adverse toxic effects (Agnew et al., 1975) and inflammatory reactions (Homsy, 1970; Dymond et al., 1970) are likely to result. In addition electrode dissolution will significantly shorten the useful life of the prosthesis. Therefore, all stimulated and implanted, non-stimulated control electrodes were examined for evidence of Pt corrosion at the electrode surface, and to evaluate the condition of the Silastic® carrier following long-term implantation (Chapter 5). These in vivo electrodes were compared with identical electrodes that had been stimulated in inorganic saline.

Finally, a series of acute studies were performed, designed to evaluate the response of the auditory nerve to various stimulus parameters - including charge density, stimulus repetition rate, charge per phase and total charge injection - with the objective of defining maximum biologically safe stimulus levels for intracochlear electrical stimulation (Chapter 6). EABRs and N1Ps were monitored prior, during and periodically following completion of acute periods of electrical

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stimulation. Stimulus induced changes in the EABR were compared with the stimulus parameter being varied. Following completion of these acute physiological experiments, the cochleas were prepared for TEM evaluation in an attempt to correlate stimulus induced physiological changes with ultrastructural changes within the auditory nerve.
4.1 Introduction.

Maximum electrochemically safe stimulus regimes have been defined for platinum electrodes. However, neural damage has been reported following electrical stimulation using stimulus parameters within these electrochemically safe limits (Chapter 3). Maximum biologically safe stimulus levels are, therefore, significantly less than the limits placed electrochemically. However, these maximum biologically safe levels have yet to be clearly defined.

The effects of long-term electrical stimulation on the surrounding tissue in general and the target neural population in particular, must be thoroughly assessed prior to the clinical application of a neural stimulator. Moreover, an appropriate animal model must be used; electrode geometry, materials, stimulus regime and stimulus site must closely model the clinical application. This chapter describes a study designed to examine the physiological and histopathological effects of long term intracochlear electrical stimulation using an electrode array and stimulator based on the Nucleus Limited Cochlear prosthesis.
4.2 Objectives.

The present study was conducted according to the following objectives:

(i) to use light microscope histological techniques to examine the effects of chronic intracochlear electrical stimulation for periods varying from 500 to 2,000 hours, and to compare these cochleas with implanted, non-stimulated control cochleas;

(ii) to use a continuous electrical stimulation program using a stimulus regime identical with, and in the upper operating range of the Nucleus Limited Cochlear prosthesis. These stimulus paradigms are designed to simulate clinical stimulation conditions that would approach worst case;

(iii) to use free-fit scala tympani electrode arrays manufactured using the same materials and techniques as used in the Nucleus Limited electrode array;
(iv) to use EABRs to periodically monitor the status of the auditory nerve during the chronic stimulation program, and to correlate changes in EABR threshold and input-output functions with cochlear histopathology.

4.3 Methods and Materials.

This study was conducted according to the Australian National Health and Medical Research Council guidelines for animal experimentation.

4.3.1 Preoperative preparation.

Ten healthy adult cats were used in the present study. All were verified as having normal auditory function by measuring the threshold for auditory brainstem responses for tone-pip stimuli at 2, 8 and 12 kHz (1 ms rise/fall time; 3 ms plateau. ABR recording techniques are described in section 4.3.4). In addition, each animal had otoscopically normal tympanic membranes.
4.3.2 Electrodes.

A scanning electron micrograph of the bipolar electrode array used in the present study is shown in Fig. 4.1. The free-fit scala tympani array was prepared by injecting Silastic® MDX-4-4210 into a 0.6 mm outside diameter (OD) mould containing two 0.3 mm wide Pt band electrodes manufactured from 99.95% Pt foil (Goodfellow Metals Ltd., U.K.). A multi-stranded Teflon insulated Pt/Ir (90:10) wire (Leico Industries Inc., N.Y.) was thermally welded to each electrode and to a Teflon insulated multi-stranded stainless steel lead wire (Davis and Geck, USA). All leadwires were encased in a 1.96 mm OD Silastic® tube and were encapsulated with Silastic® MDX-4-4210. The leadwire system was approximately 150 mm long. Low porosity Teflon felt (USCI®) discs were attached to the electrodes 6 mm from the tip using Silastic® medical adhesive Type A. The disc was included to allow fibrous tissue to seal the round window in order to minimize the entry of infection (Clark et al., 1980). In addition, two squares of Dacron® mesh (USCI®) were attached to the leadwire in order to assist the fixation of the electrode during surgery.
Figure 4.1  SEM micrograph of the bipolar electrode array used in this study. Pt, platinum electrode; S, Silastic®. Bar: 1 mm
4.3.3 Surgery.

The animals, whose weights were in the range 2.3-4.9 kg, were anaesthetized with an intra-peritoneal (i.p.) injection of ketamine hydrochloride (18 mg/kg; Ketalar®) and xylazine (3.8 mg/kg; Rompun®). Thereafter anaesthesia was maintained through intra-muscular (i.m.) injections as required.

All surgery was performed by an ENT surgeon using sterile operative techniques. The bulla cavity was exposed using a dorso-lateral approach. The ridge between the parietal and occipital bones was exposed down to the triangular area occupied by the caudal margin of the external auditory meatus, the posterior root of the zygoma and the styloid process. This triangular region was drilled in a dorso-lateral direction to expose the round window. A small inferiorly based incision was made in the round window membrane using a sterile 25 G needle, and the free-fit array was inserted a distance of 5 to 6 mm along the basal turn. The Teflon felt disc lay against the bony margins of the round window. Care was taken to ensure that the field was free of blood during the insertion, and that movement of the electrode array was kept to a minimum so as to reduce the possibility of electrode insertion trauma.
A 0.5 ml solution of cloxacillin sodium (0.1%; CSL) and ampicillin sodium (0.1%; CSL), was placed in the bulla cavity prior to the closure of the wound. The leadwire was then anchored to the bone overlying the bulla using dental cement (Paladur®). Care was taken to ensure that the dental cement remained well away from the round window. The leadwire was again anchored by looping it through two holes drilled into the frontal sinus and applying dental cement. The leadwires were brought through the skin via two small stab incisions.

Both cochleas of each animal were implanted, one side being electrically stimulated while the other side served as an implanted, non-stimulated control. Following surgery, each animal received an injection of 4 ml of antibiotic solution i.m., and had ampicillin sodium orally for five days with a dose of 100 mg/day.

4.3.4 ABR & EABR stimulus and recording techniques.

Block diagrams of the ABR and EABR stimulus generation and recording techniques are illustrated in Appendix I.

Tone pips were produced under computer control by generating trapezoidal electric pulses (1 ms rise/fall, 3 ms plateau; Hewlett-Packard 8010A) to shape an electrical sinusoid (Datapulse 410). This electrical waveform (2.0
v p-p) was fed into a sound attenuated, electrically shielded room via an attenuator. Acoustic stimuli were presented free-field from a Richard Allen DT-20 loudspeaker placed 0.1 m from the ipsilateral pinna. The acoustic stimuli were calibrated by replacing the animal with a half-inch condenser microphone (Brüel and Kjaer 4134) positioned in approximately the same position as the pinna. The output of the microphone preamplifier (Brüel and Kjaer 2615) was fed into a measuring amplifier (Brüel and Kjaer 2806) which had been previously calibrated using a piston phone (Brüel and Kjaer 4220). The maximum peak pressure (pSPL, re 20 µPa) was determined for each tone pip. Frequency analysis was performed by feeding the AC output of the measuring amplifier into an audio frequency spectrometer (Brüel and Kjaer 2112) and recording its output on a chart recorder (Brüel and Kjaer 2300). Tone pip intensities were varied from 20 to 80 dB SPL. ABR thresholds were determined at 2, 8, and 12 kHz, and compared with the laboratory standards (2 kHz, 20 dB pSPL; 8 kHz, 16 dB pSPL; 12 kHz, 30 dB pSPL). Animals having thresholds 15 dB above these levels for two test frequencies were not accepted for this study. During recording the contralateral ear was blocked with an ear mould compound (Otoform®).
Charge balanced, biphasic current pulses were used to evoke the RABR. These electrical stimuli were produced under computer control from an "in house" constant current stimulator which was electrically isolated from the recording circuitry via opto-isolators. The current amplitude was controllable over a 0 - 3 mA (peak) range and the pulse width set to 160 us per phase. Monitoring the stimulus current was performed through isolation amplifiers (AD289J).

Brainstem responses were recorded differentially (DAM - 5A Differential Preampl, WP Instruments Inc.) using subcutaneous (s.c.) needle electrodes (ipsilateral mastoid +ve, vertex -ve, contralateral mastoid ground). The responses were amplified by a factor of $10^5$ and band-pass filtered (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 analogue to digital (A/D) converter and sampled at 10 kHz for a period of 12.5 ms following stimulus onset. Stimuli were presented at 33/s and 500 responses were averaged and stored on disk for subsequent analysis. For RABR recordings the preamplifier was modified to improve the common mode rejection at high frequencies and to therefore improve the recovery time of the amplifier following the electrical artefact. In addition, these recordings were made with the preamplifier set at wide-band (0.1 Hz to 30 kHz). In order to avoid ringing in
subsequent filter sections the electrical artefact was
suppressed using a sample and hold circuit. The output
of the amplifier was sampled just before stimulus onset
and held at that level for the duration of the stimulus.

RABRs were recorded immediately following
surgery, periodically during the continuous stimulation
program, and just prior to sacrifice. RABR thresholds
and input-output functions (amplitude of the evoked
response versus stimulus current) were determined by
measuring wave III (2.2 - 2.5 ms) of the RABR waveform.

4.3.5 Electrical stimulation program.

Following a ten day postoperative recovery
period, each animal commenced a program of continuous
electrical stimulation, using a biphasic constant current
stimulation regime with a pulse width of 200 us/phase at
a rate of 500 pulses per second. The electrodes were
shorted between stimulus pulses. A block diagram of the
portable stimulator is shown in Fig. 4.2. Its design
and output waveforms were based on the current stimulator
used by the Nucleus Limited Cochlear prosthesis.
Although the stimulator was not capacitively coupled the
design featured well balanced biphasic current pulses
having measured charge asymmetries between phases of 0.01
- 0.1%. The circuitry used to measure the amount of
charge imbalance in these stimulators is shown in
Appendix II. An example of the typical charge imbalance
for these stimulators is also shown in the appendix.

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The current level chosen for long-term electrical stimulation was determined behaviourally for each animal by selecting a current level midway between threshold and a current producing an aversive response. This was confirmed to be suprathreshold by EABR recordings. These EABR data indicated that stimulus levels were in the range 3-12 dB above threshold. In this study, stimulus currents varied from 0.5 to 0.9 mA; corresponding charge densities varied from 18 to 32 nC.cm⁻² geom. per phase. The stimulator was placed in a harness worn by the cat. The output of the stimulator was connected to the percutaneous electrode leads. This allowed the animal to be continuously stimulated without restricting its movements.

Each animal in this study was monitored at least once each day, seven days per week. The stimulus current and electrode voltage waveforms were recorded to allow the impedance of the stimulating electrodes to be determined (Chapter 5). The amplitude of the stimulus current was adjusted if necessary. The battery voltage was also periodically monitored and the battery changed as required.

Although the majority of animals were continuously stimulated, a few animals had their stimulation program interrupted due mainly to lead wire breakages. These were generally repaired within 24 hours.
Figure 4.2 Block diagram of the biphasic current source stimulator used in the present study. This portable stimulator was powered by a single 6 V battery and was placed on a harness worn by the cat.

4.3.6. Histological evaluation.

(a) Tissue preparation.

On completion of the electrical stimulation program each animal was deeply anaesthetized with an i.p. injection of pentobarbitone (200 mg; Euthatal®) and perfused intra-arterially with 500 ml of a solution containing 200 units of heparin (CSL) in inorganic saline, buffered to a pH of 7.2 with 0.1M cacodylate. This was followed by 300 ml of a solution containing 2% paraformaldehyde and 3.5% glutaraldehyde in 0.1M
cucodylate buffer (Glazert, 1975). Both solutions were at 20 °C. The temporal bones were removed, trimmed, and the otic capsule thinned with a diamond paste burr. The intracochlear electrodes were removed from the round window and placed in distilled water for subsequent analysis of the electrode surface under a SEM (Chapter 5). A small hole was drilled into the apical turn of the cochlea and the oval window was opened.

Cochleas were then decalcified in 14% EDTA in neutral buffered formalin, dehydrated in ethanol and embedded in Spurr's resin (Agar Aids).

Cochleas were sectioned at a thickness of 3 um using a Reichert-Jung Autocut® microtome. Sections every 120 um were collected and stained with haematoxylin and eosin.

Histological sections were examined under a light microscope (Dialux 20: Leitz) using a magnification range of 40 to 1000. Using this tracer series, structures within the cochlea, including the electrode array, were determined using the graphic reconstruction technique of Guild (Schuknecht, 1953). The distance between pillar cells in adjacent turns of each histological section were measured. These measurements, together with the known distance between adjacent sections, were used to graphically reconstruct the cochlea (Fig. 4.3). The spatial relationship of cochlear structures, together with the electrode array, were then determined.
Although the intracochlear electrodes were removed prior to the histological preparation of the temporal bone, the fibrous tissue capsule that had surrounded the array enabled the site of the electrodes to be accurately located within the scala tympani.

(b) Spiral ganglion cell density.

Spiral ganglion cell densities were calculated for ganglion cell populations within 1 mm of the bipolar electrodes. On the basis of previous physiological data, spiral ganglion cells within this region would be electrically excited using mid-dynamic range stimulus currents (Black et al., 1985; Fig. 2.19).

The spiral ganglion cell density was determined for each section by positioning Rosenthal's canal under a 10 x 10 square eyepiece graticule at a magnification of 400. The section was positioned so Rosenthal's canal occupied as much of the area under the graticule as possible. The number of grids within the canal region were determined, together with the total number of spiral ganglion cells lying within those grids. Only cells containing a nucleus were counted. Thus, cell densities (cells/mm²) were determined for all ganglion cell populations lying within 1 mm of the electrode pair. The average and standard deviation for each cochlea was determined.
When examining the slides the investigator had no knowledge of whether the section was from a stimulated or control cochlea.

--- ORGAN OF CORI

----- SPIRAL GANGLION

1 mm

COCHLEA SECTION

3200 µm
2700 µm
2200 µm
1700 µm
1200 µm
700 µm
200 µm

Figure 4.3 Diagram illustrating the technique of graphic reconstruction of the cat cochlea and location of the intracochlear electrode array.
(c) Inflammation reaction.

An independent investigator graded the degree of inflammation in each cochlea on the basis of the amount of polymorphonuclear and mononuclear leukocytes observed, their spread throughout the cochlea, and the thickness and maturity of the fibrous tissue reaction. The degree of inflammation was graded from no reaction (I) to severe (V). When examining the slides the investigator had no knowledge of whether the slide was from a stimulated or control cochlea.

(d) Statistical analysis.

Mean spiral ganglion cell densities for stimulated cochleas were compared statistically with control cochleas using a multiple regression analysis. This statistical analysis was also used to compare mean ganglion cell densities with the degree of inflammation.

4.4 Results.

4.4.1 Cochlear histopathology.

Table 4.1 contains a summary of the histopathological results, inflammation grading, stimulus current levels and stimulation times for the 20 cochleas in this study. Electrical stimulation times varied from 424 to 2029 hours and implantation times varied from 32 to 113 days.
(a) General cochlear histopathology.

A moderate to severe inflammatory reaction was observed in five of the 20 cochleas examined (inflammation grades III - V). The remaining cochleas exhibited reactions from mild to completely absent (inflammation grades I - II). The degree of inflammation was not related to the degree of electrical stimulation.

Mild inflammation, observed in 15 of the 20 cochleas examined (e.g. 98R, Fig. 4.4), was occasionally associated with an eosinophilic exudate in the scala tympani of the basal turn. In some cases exudate had spread to the middle and apical turns of both the scala tympani and scala vestibuli. In general a mild inflammatory reaction did not result in extensive hair cell loss or atrophy of the organ of Corti. For example, the viability of hair cells in the stimulated cochlea of cat 98 was demonstrated following 1800 hours of continuous electrical stimulation. With the control ear masked with Otoform®, ABR thresholds for tone pip stimuli at 5, 8 and 15 kHz (1 ms rise/fall time; 3 ms plateau), were within the normal range. This finding, although not commonly observed in the present data, nevertheless highlights the biocompatible nature of the implanted electrode array and the stimulation regime, as hair cell loss is a sensitive index of cochlear pathology.
Table 4.1

Summary of the histopathological results for the 20 cochleas in this study.
Summary of the first part of the results for the 20 chickens in this study

Bone growth and mineral content: * = weak, + = moderate, ++ = moderate, +++ = moderate. Varicol results from previous studies.

- = moderate loss, ** = severe loss, *** = severe loss. Condition of organs of control group normal. 

<table>
<thead>
<tr>
<th>Cocks</th>
<th>Stains</th>
<th>Score</th>
<th>Growth</th>
<th>Bone</th>
<th>Stomach</th>
<th>Organs of</th>
<th>Middle</th>
<th>Middle</th>
<th>Organs</th>
<th>Condition</th>
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Two cochleas showed no apparent inflammation reaction at all (e.g. 134R, Fig. 4.5). It was unlikely that the fibrous tissue was removed during removal of the electrodes from the cochleae, as subsequent SEM analysis of these electrodes showed no evidence of fibrous tissue (Chapter 5).

With more severe inflammation, there were significant numbers of polymorphonuclear and mononuclear leukocytes, and more pronounced exudate. The increased leukocyte population was generally associated with an increased fibrous tissue reaction. The greater degrees of inflammation were associated with more extensive loss of hair cells and atrophy of the organ of Corti (e.g. 101R, Fig. 4.6). The loss of dendrites was closely associated with atrophy of the organ of Corti.

Electrode insertion trauma was noted in three cochleas. In all cases the electrode had fractured the osseous spiral lamina resulting in a moderate to severe spiral ganglion cell loss localized to the site of trauma. In two cases, the damage to the osseous spiral lamina was associated with new bone growth (e.g. 108R, Fig. 4.7). Surviving ganglion cells in the traumatized region appeared histologically normal at the limits of the light microscope, indicating that the degenerative processes had stabilized.

4-21
Fibrous tissue formation, as a result of the intracochlear implant, was seen in 18 of the 20 cochleas. The fibrous tissue varied from a fine capsule surrounding the electrode, to a moderate reaction within the scala tympani and extending to the middle turn. In a number of cases, fibrous tissue adjacent to the endosteal lining of the cochlea was associated with new bone (e.g. 107L, Fig 4.8). The extent and density of the fibrous tissue reaction was not related to intracochlear electrical stimulation.

New bone growth was recorded in nine of the 20 cochleas examined in this study. In two of the nine cases new bone formation was associated with trauma to the osseous spiral lamina during the insertion of the electrode array (e.g. 108L, Fig. 4.7). In these examples the new bone was restricted to a localized region associated with the fractured osseous spiral lamina. Little new bone was observed in the scalae. In the remaining seven cases the new bone formation did not appear to be associated with trauma to cochlear structures. In these seven cases, the new bone appeared to originate from the endosteal lining of the osseous spiral lamina and spiral ligament of the scala tympani, and was always associated with a fibrous tissue reaction within the scala (e.g. 101R, Fig. 4.6). The extent of the osteogenesis was not, however, associated with the
degree of fibrous tissue within the scala tympani or the
degree of inflammation (Table 4.1). In contrast, there
was a good correlation between new bone within the scala
tympani and peripheral cochlear pathology, i.e., atrophy
of the organ of Corti and degeneration of dendrites in
the region of the osteogenesis (e.g., Table 4.1: 107L, Fig.
4.6; 107L, Fig 4.8). In eight of the nine cochleas, new
bone had replaced less than 5% of the basal turn of the
scala tympani.

Osteogenesis was not found to be related to
intracochlear electrical stimulation, as four of the nine
cochleas exhibiting new bone growth were nonstimulated
control cochleas. Moreover, the most extensive new bone
formation was recorded in control cochlea 98R.

(b) Histopathology of the organ of Corti.

The condition of the hair cells, and the organ of
Corti in general, varied from normal to complete atrophy
throughout all turns of the cochlea. The extent of
atrophy was related to the severity of the inflammation
reaction, and was generally more extensive in the lower
basal turn close to the round window. Loss of hair
cells and atrophy of the organ of Corti were not
associated with electrical stimulation per se (e.g., Table
4.1: 98R, Fig. 4.4; 107L, Fig. 4.8; 99R, Fig. 4.9).

4-23
In a number of cochleas exhibiting minimal inflammation reactions (Grade I), atrophy of inner and outer hair cells was observed in the absence of any other pathological changes (Table 4.1). Generally the hair cell loss was restricted to the basal turn. However, in three cochleas the hair cell loss had extended to all turns, while the support cells of the organ of Corti had remained normal (e.g. 134R, Fig. 4.5). Although there was some localized dendrite loss associated with the hair cell loss, the majority of dendrites in these cochleas appeared histologically normal, and in normal populations.

(c) Spiral ganglion histopathology.

The mean spiral ganglion cell density did not diminish with increasing stimulation time (Fig. 4.10 a). Statistical analysis of the data showed no significant difference between control and stimulated spiral ganglion cell densities. However, the comparison of ganglion cell density and the degree of inflammation was highly significant (Table 4.2). The correlation between cell density and the degree of inflammation is illustrated in Fig. 4.10 b.
Microscopic examination of cochleas with little or no inflammation (i.e. grades I and II), revealed histologically normal spiral ganglion cells throughout the cochlea (e.g. 98R, Fig. 4.4; 134R, Fig. 4.5; 99R, Fig. 4.9). These spiral ganglion cells typically displayed a distinct round nucleus occupying the centre of the perikaryon, and containing a prominent nucleolus. A Schwann cell and a thin myelin sheath generally enveloped the perikaryon (e.g. 107L, Fig. 4.11a; 99R, Fig. 4.12b). Although moderate to severe inflammation was associated with a significant loss of spiral ganglion cells, the majority of remaining cells appeared normal at the level of the light microscope (e.g. 107L, Fig. 4.11b; 101R, Fig. 4.12a). Occasionally, residual ganglion cells in various stages of degeneration were observed adjacent to apparently normal ganglion cells (e.g. 101R, Fig. 4.12a). Degenerating ganglion cells typically exhibited a dark stained cytoplasm with no clear nucleus or nucleolus, as well as large vacuoles that tended to displace the nucleus, if present, to one side of the perikaryon.
(a) Basal turn of cochlea 98R. This cochlea was electrically stimulated for 2029 hours and had an inflammation grade of 1. Inner and outer hair cells appeared near normal throughout all turns. Note the fibrous tissue reaction to the presence of the scala tympani electrode array.  

(b) Spiral ganglion cells in the basal turn of cochlea 98R.  

e, electrode tract.
Figure 4.5  
(a) Basal turn of cochlea 134R. This cochlea was stimulated for 568 hours and had an inflammation reaction of I. Although the organ of Corti was present in all turns, inner and outer hair cells were absent. There was no fibrous tissue reaction associated with the electrode array. x30. 
(b) Spiral ganglion cells adjacent to the stimulating electrodes. x350.
Figure 4.6  (a) Basal turn of cochlea 101R. This cochlea was stimulated for 1115 hours, and had an inflammation reaction of IV. Hair cells were absent throughout this cochlea, and the organ of Corti was present only in a small region of the basal-middle turn. The dendrite and spiral ganglion populations were severely reduced in all turns. New bone was associated with the endosteal lining of the osseous spiral lamina and the spiral ligament of the scala tympani.  x30.  
(b) Residual spiral ganglion cells adjacent to the stimulating electrodes.  x350.  
e, electrode tract; ex, exudate; n, new bone.
Figure 4.7

(a) Basal turn of cochlea 108R. This cochlea was stimulated for 1189 h and had a grade III inflammation reaction. Polymorphs were present in the scala tympani and a pronounced exudate had spread throughout all turns of the cochlea. In addition, the electrode array had fractured the osseous spiral lamina during insertion, resulting in new bone formation. Hair cells and the organ of Corti were absent throughout all turns of the cochlea. x30.

(b) Residual spiral ganglion cells in the basal turn. x120.

e, electrode tract; ex, exudate; n, new bone.
(a) Basal turn of cochlea 107L. This cochlea exhibited extensive new bone growth. The new bone was adjacent to the endosteal lining of the osseous spiral lamina, in association with a general fibrous tissue reaction within the scala. Note the loss of the organ of Corti and peripheral dendrites opposite the osteogenesis. This cochlea was an unstimulated control. x30.

(b) Spiral ganglion cells within the basal turn. x120.

n, new bone.
Figure 4.9  (a) Basal turn of cochlea 99R. This cochlea was stimulated for 1011 hours, and was classified as having grade II inflammation. The organ of Corti appeared normal throughout all turns of this cochlea. The fibrous tissue reaction associated with the scala tympani electrode array was typical of the majority of cochleas. x30.

(b) Spiral ganglion cells in the basal turn adjacent to the electrode array. x350.
Figure 4.10  The mean spiral ganglion cell density for each cochlea in this study.

(a) Stimulated cochleas (■) have been plotted according to their total stimulation times. Control cochleas (□) have been plotted adjacent to their stimulated pair.

(b) Same data as in (a) replotted according to the inflammation reaction grade of each cochlea. Vertical bars in (a) indicate the standard deviation.
Figure a: Mean cell density (cell/mm²) vs. Stimulation Time (hours)

Figure b: Mean cell density (cell/mm²) vs. Inflammation Grade

- Stimulated
- Control

4-39
Table 4.2

Multiple linear regression analysis of mean spiral ganglion cell densities.

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<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
<th>% of total variance</th>
<th>t-Value (df-17)</th>
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<tr>
<td>Electrical stimulation</td>
<td>2</td>
<td>1.4</td>
<td>0.93 NS†</td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>5</td>
<td>61.1</td>
<td>-5.32 p&lt;0.01</td>
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</table>

† NS, not statistically significant.
Figure 4.11  (a) High power light micrograph of Type I spiral ganglion cells in the basal turn of control cochlea 107L. These ganglion cells appeared normal at the limits of the light microscope. All ganglion cells are enveloped by a thin myelin sheath (M). Schwann cells (S) can also be readily seen. The central projecting axons have a thicker myelin sheath than the perikaryon.  
x1200

n, node of Ranvier.

(b) Spiral ganglion from a region of basal turn 107L, where a localized ganglion cell loss was associated with organ of Corti and dendrite loss (see Fig. 4.8). Although the ganglion cell population was reduced by 50%, the residual ganglion cells appear histologically normal. The degenerative process took place some time prior to sacrifice, as there is no evidence of degenerative debris.  
x1200.
Figure 4.12 (a) Spiral ganglion from basal turn of cochlea 101R. Although there was significant spiral ganglion cell loss associated with a grade IV inflammation reaction, the majority of residual ganglion cells appear normal. One cell contains a large vacuole (V) on the peripheral side of the perikaryon. Although a Schwann cell (S), and remnants of a myelin sheath are still present, the morphology of this cell suggests that it is degenerating. x1200.

(b) Spiral ganglion from the basal turn of cochlea 99R. This cochlea was stimulated for 1011 hours and had an inflammation grade of II. Spiral ganglion cells appear normal at the limits of the light microscope. x1200. M, myelin sheath; S, Schwann cell.
4.4.2 Electrically evoked auditory brainstem responses.

EABR input-output functions for all animals in this study are shown graphically (Figs. 4.13, 4.14 & 4.15). Immediate postoperative input-output functions were similar for all animals in this study (e.g. cat 112, day 0, Fig. 4.13). A low gradient limb of the input-output function displayed a low threshold, typically between 50 uA - 0.1 mA, and a large dynamic range. A second high-gradient limb of the input-output function was generally observed for stimulus currents greater than 0.5mA - 0.75 mA. In these examples, stimulus current is expressed logarithmically to highlight the two distinct limbs in the input-output function. The low gradient limb could be readily masked with low intensity broad band white noise, and was identified as basically electrophonic in origin (Black et al., 1983b).

Three distinct trends in EABR input-output functions were observed during the chronic stimulation program. First, a group represented by cats 98, 99, 107, and 112 (Fig. 4.13), maintained throughout the stimulus period - two distinct limbs in their EABR input-output functions. EABRs from these animals therefore remained similar to their immediate postoperative response. Thresholds remained constant (e.g. 107, Fig. 4.13), or increased slightly with stimulation time (e.g. 98, Fig. 4.13), to a maximum of 0.1 mA. These stable EABR input-output functions have been designated Type I.
A second group, represented by cats 109, 117 and 134 (Fig. 4.14), showed progressively increasing thresholds to levels of typically 0.3 to 0.5 mA, and a significant reduction or total loss of the first, low-gradient limb of the input-output function (e.g. 134, Fig. 4.14). In some cases EABR data from these cochleas also exhibited a shift in the second, high gradient limb, towards lower stimulus current levels (e.g. 117, Fig. 4.14). EABR input-output functions exhibiting these characteristics have been designated Type II. The third group of animals, cats 101, 108 and 132 (Fig. 4.15), showed a significantly greater elevation in threshold compared with animals exhibiting Type II EABR input-output characteristics. Threshold currents were typically elevated to 0.7 mA (e.g. 108, Fig. 4.15). Associated with the elevation in threshold, was a complete loss in the low-gradient limb and, in contrast with animals showing Type II trends, a significant reduction in the gradient of the second limb of the input-output function (e.g. 108, day 74, Fig. 4.15). More extensive changes in input-output functions such as these, have been categorized as Type III.

Although some animals showed significant shifts in EABR threshold, these data indicate that all animals were stimulated throughout their stimulus programs (note that EABRs were recorded at 100 us per phase while the animals were stimulated at 200 us per phase).
Figure 4.13 Chronically recorded EABR input-output functions for four cats exhibiting Type I EABR characteristics. Note the stable thresholds and the presence of the low gradient limb of the i/o functions throughout the chronic recording period. Recording days are days post-surgery. Dashed lines indicate recordings made prior to the commencement of chronic stimulation.
Figure 4.14 Chronically recorded EABR input-output functions for three cats exhibiting Type II EABR i/o characteristics. Note the increased thresholds associated with a loss of the low gradient limb of the i/o function.
Figure 4.15 Chronically recorded EABR input-output functions for three cats exhibiting Type III EABR i/o characteristics. These three animals showed a dramatic increase in threshold, together with a total loss of the low gradient limb and a significant loss of the high gradient limb of the i/o function.
4.5 Discussion.

The histopathological and physiological results obtained from this study indicate that carefully controlled, charge balanced stimulation regimes, operating well within the electrochemically safe limit of 300 uC.cm\(^{-2}\) geom. per phase, do not adversely affect spiral ganglion cell populations close to the stimulating electrodes, or the cochlea in general. The work does, however, highlight the adverse effect of infection on the neural population of the cochlea. In addition the present research has demonstrated a correlation between changes in EABR threshold and supra-threshold responses with cochlear histopathology.

The minimal inflammatory reaction associated with long-term scala tympani electrode implantation, illustrated in the present study, reflects the biocompatible nature of both the electrode array and the electrical stimulus. The demonstrated biocompatibility of the electrode array is consistent with previous findings that have investigated the biocompatibility of both Silastic® MDX-4-4210 and Pt; the materials used in the manufacture of the banded scala tympani electrode. The tissue response to chronic implantation of Pt has been evaluated by several investigators. For example, Dymond et al. (1970) reported a minimal tissue reaction following two months implantation of Pt in the brains of cats, and concluded that this metal can be considered
non-toxic in vivo. The biocompatible nature of Pt has been confirmed following its implantation in scala tympani of a variety of laboratory animals for implant periods of up to 14 months (Clark, 1977; Burgio, 1985). The biocompatibility of Silastic® MDX-4-4210 has been evaluated in both muscle and cochleae of experimental animals. Clark and his colleagues (Clark et al., 1983) compared the tissue reaction of Silastic® MDX-4-4210 with a variety of other Silastic® biomaterials. The degree of tissue reaction was evaluated by the polymorphonuclear and mononuclear leukocyte reactions and the degree of fibrous tissue associated with the implant. The materials were implanted in the muscle of rats for a two week period. The results indicated that Silastic® MDX-4-4210 evoked a minimal tissue reaction and could therefore be considered biocompatible. A subsequent study emphasized the biocompatible nature of this material following implantation in the scala tympani and muscle of cats for a 16 week period (Shepherd et al., 1984). Another prosthesis group has also confirmed the biocompatibility of Silastic® MDX-4-4210 following chronic scala tympani implantation (Leake et al., 1985).

In the present study, it was significant that the tissue reaction adjacent to the stimulated electrodes was similar to the reaction adjacent the control electrodes. This observation suggests that the electrical stimulus
per se can be considered biocompatible, as an adverse electrical stimulus is likely to evoke an extensive inflammatory reaction (Pudenz et al., 1977). Moreover, the present results suggest minimal irreversible electrochemical reactions were occurring at the electrode-tissue interface as a result of the charge injection process. Electrochemical reactions such as metal dissolution and hydrogen and oxygen production would result in long-term chemical irritation of the adjacent tissue, evoking a severe inflammatory reaction which would include an acute inflammatory component (Coleman et al., 1974; Loeb, 1983). One may also conclude from the present results that the biocompatibility of the Silastic® MDX-4-4210 remains unchanged during long-term electrical stimulation of adjacent Pt electrodes. These histopathological observations have been supported by SEM analysis of both the stimulated and control scala tympani electrodes (Chapter 5).

There are a number of previous investigations that have evaluated the histopathological response to long-term intracochlear electrode implantation with and without electrical stimulation. The majority of these studies have involved the use of experimental animals, however there are a number of reports of the histopathological response to cochlear implantation in human (Johnsson et al., 1982; Linthicum and Galey, 1983; Galey, 1984). Although there are consistent findings
between the present research and previous work, there also exist a number of conflicting histopathological observations. These differences probably reflect variations in experimental technique and materials. The underlying reasons for these variations must be resolved before definitive statements regarding the safety and efficacy of cochlear prostheses have involved the use of experimental animals, however there are a number of reports of the histopathological response to cochlear implantation in human (Johnsson et al., 1982; Linthicum and Galey, 1983; Galey, 1984). Although there are consistent findings between the present research and previous work, there also exist a number of conflicting histopathological observations. These differences probably reflect variations in experimental technique and materials. The underlying reasons for these variations must be resolved before definitive statements regarding the safety and efficacy of cochlear implants can be made.

There is now a significant body of evidence to indicate that the cochlea can tolerate long-term implantation of a scala tympani electrode array without adverse histopathological consequences. For example, two of the 20 cochleas in the present study, in addition to a number of cochleas in previous studies (Simmons, 1967; Clark, 1977), have shown no foreign body reaction to chronic scala tympani implantation. Although this is not a common observation, it nevertheless illustrates
that there are a variety of biomaterials that can be extremely well tolerated when implanted within the cochlea.

The most common histopathological response observed in the present study was the formation of a thin fibrous tissue capsule enveloping the electrode array, externalizing the implant from the scala tympani. Areolar tissue was usually associated with the fibrous tissue capsule, and generally extended throughout the lower basal turn scala tympani (e.g. 98R, Fig 4.4). Frequently associated with this reaction was a loss of inner and outer hair cells overlying the electrode array. Hair cells more apically were usually present except in cases of adverse inflammation as a result of infection. Outer hair cells appeared more susceptible to degeneration than inner hair cells. Similar observations have also been made by other investigators (e.g. Leake-Jones and Rebscher, 1983). Support structure of the organ of Corti adjacent to the implanted electrode exhibited less atrophy than the sensory cells, and were present in 10 of the 20 cochleas. More apically, this structure was present in 18 of the 20 cochleas examined. In the remaining two cochleas the organ of Corti had completely atrophied throughout all turns of the cochlea. These pathological changes have been attributed to a generalized infection as both cochleas exhibited severe inflammation (101R, grade IV; 4-54
Significantly, hair cell and organ of Corti atrophy were not related to the degree of electrical stimulation (Table 4.1). For example, of the 14 cochleas that exhibited inner and outer hair cell loss in the lower basal turn, eight were nonstimulated control cochleas.

In general, dendrite survival was closely associated with organ of Corti survival. Leake and her colleagues have also made this observation following chronic scala tympani implantation (Leake-Jones and Rebscher, 1983; Leake et al., 1985). These findings support the notion that the collapse of the organ of Corti is the precursor to anterograde degeneration of auditory nerve fibres (Kerr and Schuknecht, 1968; Schuknecht, 1974; Johnson, 1974; Otte et al. 1978). However, it must be emphasized that these are generalized observations, in a number of cases in the present study, dendrite and spiral ganglion cell loss were observed despite the presence of the support structure of the organ of Corti (e.g. 112L, Table 4.1). It is conceivable that these variations in the histopathological response reflect different pathological mechanisms. For example, the collapse of the organ of Corti may result in damage to the dendrites of the spiral ganglion, thus initiating anterograde degeneration. However, in some infections, the non-sensory cells of the organ of Corti may not be affected while neural elements
within the osseous spiral lamina may degenerate.

Many of the histopathological responses observed in the present study are similar to those of several previous investigations. Significantly, these common findings have been derived from studies that have used a variety of electrode designs and/or biomaterials. Simmons (1967), implanted fine enamel coated stainless steel wires for distances of up to 10 mm along the scala tympani in the cat. Except in the case of infection or electrode insertion trauma, these cochleas tolerated the presence of foreign bodies very well. Infection resulted in extensive degeneration of both the organ of Corti and the spiral ganglion, whereas trauma to the basilar membrane resulted in a complete loss of the organ of Corti and a 50% loss of the spiral ganglion in a region local to the damage. Regions of the cochlea more apical to the trauma appeared normal. In cochleas without infection or insertion trauma, the majority of hair cells appeared normal. Indeed, normal CAP and CM thresholds could be obtained from one animal six months following implantation of the electrode. This finding is similar to the observation in the present study of normal ABR thresholds obtained from cat 98R following 1800 hours of electrical stimulation.

Schindler and his colleagues (Schindler and Merzenich, 1974; Schindler, 1976; Schindler et al., 1977), have evaluated the histopathological response
following chronic implantation of a Silastic® electrode array moulded to closely fit the first 9 mm of the cat scala tympani. Implantation periods of up to 2.5 years have been reported. Their findings include severe hair cell loss in the basal turn adjacent to the electrode array, with less extensive hair cell loss in middle and apical turns. The support cells of the organ of Corti were generally present throughout the cochlea. Furthermore most neurones and spiral ganglion cells adjacent to the electrode array survived, and their functional viability was confirmed just prior to sacrifice of the animal. Significantly, these authors indicated that the pathological changes were usually observed within the first four to six weeks following implantation, after which the cochlea was stable. This long-term stability was also observed in the present study.

The only significant difference between the findings of the present study and those of Schindler and colleagues, is the more extensive hair cell loss observed in their study. It is possible that this discrepancy is due to the difference in size of the two electrode arrays. A large electrode array displacing a significant amount of perilymph and lying close to the basilar membrane, may have a more adverse effect on hair cell survival than a smaller, distally located electrode. Indeed, more recent work from this implant
group has shown less damage to the organ of Corti adjacent to the implant following the implantation of a slightly smaller moulded array (Leake-Jones and Rebscher, 1983; Leake et al., 1985). These authors suggest that impairment of perilymph circulation - associated with the larger electrode - may have contributed to the greater hair cell loss.

This new moulded scala tympani array, manufactured from Silastic® MDX-4-4210, evoked a similar tissue reaction to that observed in the present study (Leake et al., 1985). The tissue capsule was reportedly highly collagenous with fibroblasts being the major cellular component. Although, in favourable examples, there was no observable chronic inflammation, a few macrophages were generally present adjacent to the implant. Significantly, in cases where the electrode had traumatized the basilar membrane or osseous spiral lamina, an adverse inflammatory and foreign body reaction was observed. Moderate to severe inflammatory reactions were also observed in the three cochleas that exhibited insertion trauma in the present study. It is possible that these inflammatory reactions were in response to structural damage, although one cannot rule out the possibility of previous infection.

In accordance with previous findings, this group has reported severe localized neural degeneration associated with basilar membrane and osseous spiral
lamina damage (Schindler and Bjorkroth, 1979; Leake-Jones and Rebscher, 1983; Leake et al., 1985). The degeneration of ganglion cells occurred soon after the damage - usually less than four weeks. Damage to the osseous spiral lamina, and hence to the myelinated dendrites, resulted in more rapid degeneration than damage to the basilar membrane and organ of Corti (Leake-Jones and Rebscher, 1983; Leake et al., 1985).

In a series of tissue tolerance studies, Clark and colleagues (Clark et al., 1975; Clark, 1977), examined the histopathological response to a variety of electrode materials using both round window scala tympani insertion, and a basalward insertion along the scala vestibuli via a fenestration in the otic capsule. These studies indicated that electrode insertion via the round window could result in minimal damage to the cochlea despite implantation periods of up to 59 weeks. In a number of cochleas the hair cell and spiral ganglion population adjacent to the implant appeared normal. These authors reported greater trauma associated with an apical to basal scala vestibuli insertion. These studies also demonstrated that damage to the osseous spiral lamina or basilar membrane resulted in severe localized spiral ganglion cell loss. Moreover, they also found that permanent damage to Reissner's membrane resulted in a significant localized loss of spiral ganglion cells. Infection, when observed, resulted in
widespread ganglion cell loss.

Although there are consistent findings between the present and previous research examining the tissue tolerance to long-term scala tympani implantation, there are a number of reports that present conflicting histopathological observations. Miller and his colleagues have routinely demonstrated extensive histopathological changes following chronic scala tympani implantation. Extensive new bone growth, atrophy of the organ of Corti and widespread degeneration of the spiral ganglion are commonly observed (Sutton et al., 1980; Pfingst and Sutton, 1983; Sutton and Miller, 1983). In many cases these severe pathological changes were not restricted to regions of the cochlea adjacent to the electrode array. The most significant finding reported is the observation of ongoing pathological changes evident for periods of greater than 24 months post-implantation. This finding is in contrast to the present research and a number of previous studies which have reported long-term stability following cochlear implantation.

There are a number of possible causes that may have contributed to these adverse histopathological responses. First, it is possible that an insertion of an array 8-12 mm along the monkey scala tympani is more traumatic than the insertion of an array 5-6 mm along the scala tympani in the cat. It has been suggested that
the cat scala tympani is more robust than the monkey (Sutton et al., 1980). If this were so, one would expect severe localized neural degeneration occurring at periods soon after the insertion of the array. This form of pathology could account for large localized differences in neural survival observed close to the electrode, however, it could not account for the long-term degeneration observed both distally and adjacent to the array. Second, the effects of acute periods of electrical stimulation, although not controlled for in these studies, may have contributed to the ongoing degenerative processes observed. There is good evidence that the type of electrical stimuli used (continuous sinusoidal), is damaging at stimulus intensities within the range used in these studies (Duckert, 1983; Duckert and Miller, 1984). Significantly, there is evidence of a greater reduction in ganglion cells adjacent to the metal electrodes, compared with the ganglion cell populations adjacent to the Silastic® carrier in these reports (Pfingst and Sutton, 1983; Sutton and Miller, 1983). Third, a number of cochleas were directly flushed with a neomycin solution just prior to implantation of the electrode array. Such a procedure produces severe histopathological changes including extensive neural degeneration and new bone growth. These changes would mask histopathological changes associated with the electrode or electrical stimulation.
(Duckert, 1983; Balkany et al., 1985). Clearly, such animal models are not appropriate when evaluating the histopathological response of the implant or electrical stimulation per se. Finally, there is evidence that unbound stannous octate - the catalyst used to cure the Silastic® elastomer used in these studies - had, on occasion, leached from the electrode array into adjacent tissue (Duckert and Miller, 1984). Such chemical irritation of the surrounding tissue would likely result in an adverse histopathological response that could be active for long periods following implantation. Stannous octate has shown severe biotoxic properties following chronic subcutaneous implantation in rats (GM Clark, unpublished data). Moreover, other investigators have also reported occasional adverse tissue responses following chronic implantation with scala tympani arrays manufactured using a similar Silastic® elastomer (Leake-Jones and Rebscher, 1983; Leake et al., 1985). The authors attributed these occasional adverse reactions as probably being due to batches of elastomer containing unbound stannous octate. It is likely that a combination of these effects contributed to the adverse histopathology observed in these cochleas.

These findings highlight the importance of manufacturing implants for animal research according to the protocols used in the production of prostheses for human use. Moreover, implants must be sterilized using
the same techniques as those used for the human prosthesis. For example, there is some evidence of variable biocompatibility results that have been attributed to contamination during autoclave sterilization (Roberts et al., 1983). Sterilization in ethylene oxide should reduce the chances of contamination. In addition to these requirements, animal surgery must be performed using sterile techniques, and the animals administered with an appropriate antibiotic regime.

The results of the present study indicates that long-term intracochlear electrical stimulation using short duration charge balanced biphasic current pulses, operating at or below 32 uC.cm⁻² geom. per phase, does not adversely effect the adjacent spiral ganglion cell population or the cochlea in general. These safe stimulus levels are well within the electrochemically safe limits for Pt electrodes of 300 uC.cm⁻² geom. per phase (Brummer et al., 1977). Significantly, a number of other studies using similar electrode materials and stimulation regimes have reported safe stimulus levels below approximately 40-50 uC.cm⁻² geom. per phase (see review, 3.7). Above this stimulus intensity neural damage reportedly increases with charge density (Pudenz et al., 1975 a&b; Agnew et al., 1975; Pudenz et al., 1977; Yuen et al., 1981; Walsh and Leake-Jones, 1982). It would therefore appear that the maximum biologically
safe stimulus levels - for short duration, charge balanced, biphasic current pulses and Pt electrodes - is significantly less than the maximum limits defined electrochemically. This observation suggests that the neural damage mechanism at these stimulus intensities are due to effects of electrical stimulation per se rather than the effects of adverse electrochemical reactions. Similar conclusions have been made by others (Pudenz et al., 1975b). One possible mechanism may simply be excessive neural activity. There are, for example, a number of physiological studies that illustrate the inability of various neural tissue to maintain homeostasis during electrical stimulation at intensities and rates greater than the physiological norm for that tissue (McCreery and Agnew, 1983; Yarowski et al., 1983). Prolonged stimulation using highly synchronous stimuli such as an electric pulse, at unnaturally high stimulus intensities and pulse rates, could place the excitable tissue under considerable metabolic stress. This insult could conceivably result in neural degeneration (Chapter 6).

A number of investigators have reported widespread stimulus induced neural degeneration at charge densities much less than the damage levels observed following stimulation with short duration biphasic pulsatile stimuli. Using continuous sinusoidal stimulus currents, significant stimulus induced damage was
observed in the cochlea following short periods of stimulation at 1 kHz, for charge densities as low as 15 uC.cm\(^{-2}\) geom. per phase - the lowest intensity tested in these studies (Duckert and Miller, 1984; Miller et al., 1985). It is likely that even lower neural damage thresholds would have been observed had lower stimulus intensities been used. Pathological changes included severe organ of Corti and spiral ganglion cell loss adjacent to the electrode, and extensive new bone growth in the scala tympani in the region of the array. These extensive stimulus induced changes were observed following just 12 hours of stimulation over a four week period. There are a number of possible explanations that may account for the adverse effects of this form of electrical stimulus. First, the electrochemical behaviour associated with this continuous stimulus is not well documented. At 1 kHz, the duration of each phase of the biphasic waveform is 1 ms, considerably longer than the pulse width recommended for pulsatile stimuli on electrochemical grounds (Lilly, 1961; Brummer et al., 1977). It is possible that much of the damage reported following stimulation with this type of waveform, is due to adverse electrochemical reactions. Indeed, continuous stimulation at lower frequencies (100 Hz) produces more severe tissue damage (Duckert and Miller, 1984). These investigators attribute this increase in damage to adverse electrochemical reactions. Such
stimulus waveforms are far from ideal electrochemically (Lilly, 1961; Mortimer et al., 1970; Brummer et al., 1977). Second, the continuous nature of the stimulus is likely to evoke significant neural activity, limited only by the absolute and relative refractory periods of the neurone, and the strength and phase of the stimulus. Miller and his colleagues (Miller et al., 1983; Miller et al., 1985), have reported temporary elevations in threshold and reductions in the suprathreshold response of EABRs following periods of acute stimulation using continuous 1 kHz sinusoidal stimuli. One mechanism underlying these temporary changes in the evoked response, suggested by these authors, may be metabolic exhaustion of the first order auditory neurones or depletion of available transmitter at synaptic junctions within the ascending auditory pathway. As discussed previously, it is conceivable that periods of prolonged activity under conditions of severe metabolic stress, could result in neural degeneration. Finally, it is possible that the continuous nature of the sinusoidal stimulus releases a greater amount of energy in the form of heat compared with transient pulsatile stimulus. This would also be likely to result in an adverse tissue response.

It is probable that there are a number of mechanisms underlying the stimulus induced neural damage reported in these studies. Certainly, physiological damage due to metabolic exhaustion would appear to be one
mechanism. However, the nature of the damage observed in these studies was not restricted to neural tissue. The histopathological response included new bone growth associated with the electrode array. Such pathology illustrates the general noxious nature of the stimulus regime. Deliberate overstimulation of pulsatile stimuli (100-200 \( \text{uC.cm}^{-2} \text{ geom. per phase} \)), or a significant charge imbalance in the biphasic pulse, has evoked similar pathological reactions (Walsh and Leake-Jones, 1982).

An important finding in the present study was that total charge injection per se was not related to neural damage. The aggregate charge in the present study was as high as 356 Coulomb. Significantly, there was no correlation between aggregate charge and the degree of spiral ganglion cell loss. This finding illustrates the long-term biocompatibility of this type of stimulus, operating at low and moderate stimulus intensities. This finding is in contrast with Agnew and colleagues (Agnew et al., 1983), who reported increased neural damage with total charge injection, at a fixed charge density of 100 \( \text{uC.cm}^{-2} \text{ geom. per phase} \). Since these authors were stimulating at a charge density known to result in neural damage, it is probable that total charge injection is related to neural damage only at charge densities above the biologically safe limit.

Our understanding of the histopathological
response to cochlear implants has almost exclusively been based on animal studies. Recently, these studies have been complimented by a number of reports describing the histopathology of human temporal bones following cochlear implantation. Such studies provide unique opportunities to evaluate the biocompatibility of these devices in human, however they must be examined with some caution due to the uncontrolled effects of previous pathology, and the duration of implantation and extent of electrical stimulation.

Johnsson and colleagues (Johnsson et al., 1982), have described the histopathology of two temporal bones taken from a patient with bilateral cochlear implants. The right cochlea of this patient contained a single Pt/Ir wire electrode that had been inserted approximately 17 mm from the round window. The patient had received this implant approximately two years prior to his death, however the device had only been used intermittently for about three months. Histological examination of this cochlea indicated that the single wire had passed along the scala tympani for the first 12 mm, after which it had deviated into the scala media and finally the scala vestibuli, damaging the spiral ligament on its course. Significantly, the basilar membrane, Reissner's membrane and the osseous spiral lamina were intact. Despite this trauma no new bone growth was observed in this cochlea; there was only a limited soft tissue reaction associated
with the electrode. Extensive sensory and neural loss was apparent throughout all turns of the cochlea, although it is not possible to differentiate degeneration due to the implant and degeneration due to the preceding pathology. The minimal tissue reaction observed in this cochlea is similar to the typical reaction observed in the present animal study.

The left cochlea of this patient contained a multichannel electrode array that consisted of five silver wires. The most apical wire was inserted approximately 20 mm with the remaining wires being spaced at about 4 mm intervals. The patient had received this implant seven years before his death, and it had been used for six to eight hours per day for approximately 27 months. This cochlea showed more extensive histopathological changes. The wire electrodes were surrounded by bone for their entire course within the scala tympani. As in the right cochlea, the array had deviated from the scala tympani approximately 12 mm from the round window. Again, the electrode tip was located in the scala vestibuli, and in this example, extensive new bone was found in the scala media and scala vestibuli local to the electrode. Significantly, regions of the membranous labyrinth showed evidence of blackening which was subsequently shown to be silver. Hydrops was also evident in this cochlea. Greater neural degeneration was evident compared with the opposite ear.
The extensive new bone present in this cochlea, could be due to several factors. First, the extensive electrode insertion trauma would - on the basis of the present and previous animal studies - be likely to contribute to the formation of new bone. Second, the adverse tissue reaction associated with the silver electrodes. Silver has long been regarded as a non-compatible metal (Pudenz, 1942; McFadden, 1969; Dymond et al., 1970). When passively implanted it produces a pronounced tissue reaction which could conceivably resolve in bone. Presumably, this metal is more corrosive under conditions of electrical stimulation. Finally, there is some evidence that the new bone formation observed in this patient may be related to etiology (Linthicum and Galey, 1983).

In a preliminary report on the temporal bone of a patient who had received a multichannel electrode array six years prior to his death, Galey (1984) described extensive electrode insertion trauma. The electrode array consisted of six individual 0.21 mm diameter Pt/Ir wires inserted along the scala tympani for distances of up to 22 mm from the round window. Only two of the six wires were found entirely within the scala tympani; the tips of three wires lay in the scala vestibuli, and one lay in the scala media. So severe was the trauma that several wires had penetrated the basilar and Reissner's membranes; some wires had reflected off the outer wall of
the scala vestibuli causing further damage to the membranous labyrinth. Associated with this trauma was evidence of extensive new bone growth within the first 10 mm of the scala vestibuli. Significantly, there was no new bone associated with the four apical electrodes distal to the trauma; these electrodes were used for electrical stimulation. Although dendrite loss appeared extensive in this cochlea, spiral ganglion survival was not assessed.

These observations are generally consistent with previous animal studies. The human cochlea can tolerate chronic electrode implantation and electrical stimulation. However, electrode insertion trauma or non-compatible implant materials can evoke adverse inflammatory reactions that typically resolve as new bone. Furthermore, the results illustrate the inadequate design of these early multielectrode arrays. More recently, arrays containing significantly thinner Pt/Ir wires within a Silastic® carrier have been shown to result in minimal trauma following insertion into the human cochlea (Shepherd et al., 1985; Franz and Clark, in press; Clifford and Gibson, in press).

It is worth noting that although there was variable, and in some cases severe, pathological responses observed in these cochleae, both patients received some benefit from their implant.
New bone within the scala tympani was reported in nine of the 20 cochleas examined in the present study. Although, in all cases the new bone occupied only a small portion of the basal turn scala tympani, it is possible - had implantation times been longer - that more extensive new bone would have been observed. New bone growth has been a common finding in both animal and human temporal bone studies.

New bone growth is considered undesirable for a number of reasons. First, as observed in the present and previous animal studies (e.g. Sutton and Miller, 1983), new bone growth generally reflected greater neural loss within the adjacent osseous spiral lamina (e.g. 107L, Fig. 4.8). In these cases it is assumed that the pathology initiating new bone growth, also caused atrophy of the organ of Corti and anterograde degeneration of auditory nerve fibres. Second, extensive new bone may significantly alter the current distribution, restricting the spread of current into Rosenthal's canal, and resulting in increased threshold stimulus currents. Third, new bone surrounding the electrode may make electrode removal and re-insertion difficult. Finally, there is concern that new bone may displace the electrode array, affecting the ability of the implant to excite discrete populations of auditory nerve.
There are a number of factors that may contribute to new bone growth within the scalae. Certainly, new bone is nearly universally observed in response to electrode insertion trauma. Rarely is damage to the basilar membrane or osseous spiral lamina resolved without osteogenesis (Simmons, 1967; Schindler and Merzenich, 1974; Clark, 1977; Schindler and Bjorkroth, 1979; Sutton and Miller, 1983; Galey, 1984; Leake et al., 1985). Damage to the spiral ligament, however, is frequently resolved without new bone (Johnsson et al., 1982; Clark et al., in press). Extensive inflammatory reactions due, for example, to infection (Clark, 1977); direct neomycin infusion of the cochlea (Pfingst and Sutton, 1983; Sutton and Miller, 1983; Duckert, 1983; Balkany et al., 1985); and adverse foreign body reactions (Johnsson et al., 1982), almost universally evoke osteogenesis. Moreover, there is evidence to suggest

+ Our limited experience with re-insertion of the banded electrode array suggests that the distal end of the original electrodes were not surrounded by new bone. In both cases, the new electrode was inserted with ease at least 5 mm further than the original electrode. These original electrodes had been implanted for 3.8 and 5.0 years, and had been stimulated for much of that time.

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that new bone is also evoked following adverse intracochlear electrical stimulation (Walsh and Leake-Jones, 1982; Duckert and Miller, 1984). Significantly, in the present study new bone was not associated with electrical stimulation. Four of the nine cochleas exhibiting new bone growth in this study were non-stimulated control cochleas. This finding highlights the biocompatible nature of the electrical stimulus used in the present study, but also serves to illustrate the damaging nature of charge balanced electrical stimuli using adverse stimulus parameters.

In the majority of cochleas in the present study, new bone appeared to originate from the endosteum lining the scala tympani. This was usually associated with a general fibrous tissue reaction within the scala. Typically, the principle site of new bone growth appeared to be the endosteum of the osseous spiral lamina overlying the myelinated dendrites of the auditory nerve, and the endosteum overlying the spiral ligament. Moreover, new bone was generally restricted to the lower basal turn scala tympani. This is a common finding in cochlear implant histopathology (e.g. Johnsson et at., 1982; Sutton and Miller, 1983), and other forms of cochlear pathology such as suppurative or meningococcal labyrinthitis (Schuknecht, 1974), and presumably reflects the major site of irritation. In the present study, there was no correlation between the extent of fibrous
tissue within the scala and the degree of new bone growth.

The relationship between fibrous tissue and the formation of new bone at the endosteal lining, reported in the present study, has also been reported by a number of other investigators (Schindler and Merzenich, 1974; Clark, 1977; Schindler and Bjorkroth, 1979; Walsh and Leake-Jones, 1982). This finding emphasizes the need to ensure minimal inflammation associated with electrode implantation. This can be achieved by using strict aseptic techniques during surgery, a clean electrode fabrication protocol, and materials of known biocompatibility.

The EABR data obtained during this study confirmed the viability of the spiral ganglion cells examined histologically. Furthermore, these data showed good correlation with cochlear pathology, illustrating the potential clinical application of this monitoring technique.

Cochleas with normal or near normal hair cell and spiral ganglion cell populations produced stable (Type I) EABR input-output functions throughout the chronic recording period. These Type I input-output functions exhibited two characteristic limbs; an initial low-gradient limb followed by a second high-gradient limb. Such input-output behaviour is characteristic of normal hearing cochleas stimulated with bipolar scala tympani.
electrodes (Yamane et al., 1981; Black et al., 1983b). Cochleas showing normal spiral ganglion populations with complete or near complete hair cell loss exhibited Type II EABR input-output characteristics which included an elevation in threshold associated with a gradual loss of the low-gradient limb. In addition, a number of cochleas exhibiting Type II characteristics also showed a shift of the high-gradient limb towards lower stimulus currents. This shift presumably indicates some form of variation in the resistance patterns within the cochlea as a result of the deafening process (Black et al., 1983b). Finally, cochleas showing spiral ganglion cell loss in addition to hair cell loss, exhibited Type III input-output characteristics including a complete loss of the low-gradient limb, a significant increase in threshold, and a reduction in the slope of the high-gradient limb. It is probable that the significantly higher EABR thresholds associated with these changes are due to a marked loss of dendrites, resulting in higher stimulus currents in order to excite more distally located spiral ganglion cells. Furthermore, the reduction in the slope of the high-gradient limb probably reflects the significant reduction in the ganglion cell population.

Although these data demonstrated good qualitative correlation with the observed cochlear pathology, there was no evidence of a quantitative correlation between the
slope of the high-gradient limb of the input-output function and the degree of spiral ganglion cell loss as reported by Smith and Simmons (1983). For example, the slope of the final input-output function recorded for cat 132 (day 37, Fig. 4.15), was greater than would be predicted by residual spiral ganglion populations. It is possible that the EABR responses for this cochlea, at the relatively high stimulus currents required to elicit a response, included a modiolar component, as there is evidence - at least in human temporal bones - of relatively large numbers of surviving auditory nerve axons in cochleas containing relatively few spiral ganglion cells (Ylikoski et al., 1981). It is also possible that discrepancies between the observations of Smith and Simmons (1983) and the present study are due to the use of different electrode geometries.

The EABR data confirmed the presence of a large dynamic range, low threshold electrophonic limb in animals with normal or near normal hair cell populations. This finding highlights the need for investigators to take into account the presence of electrophonic stimulation when electrically stimulating normal hearing cochleas. For example, Pfingst et al. (1981), described a study in which psychophysical data were compared with cochlear histopathology in normal hearing monkeys implanted with scala tympani electrode arrays. One animal had a significantly low threshold
and a large dynamic range (50.5 dB) compared with the remaining six cochleas. Subsequent histological evaluation revealed that this animal had 30% of hair cells remaining in the basal turn and 70% in more apical turns. This was a far greater hair cell population than the other cochleas. It is most likely that the dynamic range of this animal had an electrophonic component. A similar finding, illustrating the relationship between surviving hair cells and auditory neurones with EABR dynamic range, was noted in the present study (e.g. Fig. 4.13).

The initial EABR input-output function recorded from cat 108 (Fig. 4.15) in the present study, showed a relatively high threshold and a narrow dynamic range compared to the majority of animals in the study. These variations are probably due to electrode insertion trauma which damaged the organ of Corti and the osseous spiral lamina for some distance. Similar observations have been reported in primate psychophysical studies where cochleas showing evidence of electrode insertion trauma have high thresholds and reduced dynamic ranges compared with cochleas without insertion trauma (Pfingst et al., 1981).

Chronic monitoring of the status of the auditory nerve with EABR provided some insight into the temporal aspects of various cochlear pathologies. For instance, the gradual and orderly loss of the low-gradient
electrophonic limb of the input-output function, for animals exhibiting Type II EABR characteristics (e.g. cat 117, Fig. 4.14), is consistent with the progressive basal to apical hair cell loss commonly observed in cochlear pathology. In contrast, rapid and significant changes were observed in the EABR of two animals that showed extensive spiral ganglion cell loss as a result of infection (cats 108 & 132, Fig. 4.15). In both cases, the input-output functions recorded at approximately three weeks post-implantation appeared normal. However, the following recording session, two weeks later, revealed Type III EABR characteristics. Thereafter, the EABR input-output functions remained stable. These adverse changes, associated with infection, highlight two important points. First, the susceptibility of the cochlea to infection for periods shortly following cochlear implantation, and prior to the formation of an effective round window seal. This finding is consistent with histological observations of the development of the round window seal following cochlear implantation (Franz et al., 1984). Second, the extensive changes in the EABR, observed over a two week monitoring period, reflect the severe and rapid degeneration of the auditory nerve following infection. Such rapid degenerative changes are in contrast with the more gradual anterograde degenerative changes observed following physical damage to the cochlear periphery (see review, Chapter 3).
4.6 Summary.

The ability of spiral ganglion cells to survive long-term electrical stimulation is a precondition for the success of a cochlear prosthesis. In this study 10 cats were implanted bilaterally with bipolar scala tympani electrodes. One cochlear of each animal was stimulated for periods of up to 2029 hours using charge balanced biphasic current pulses. The status of the auditory nerve was monitored periodically by recording EABRs. At the conclusion of the stimulation program, spiral ganglion cell survival was assessed for both stimulated and control cochleas; comparison of the spiral ganglion cell densities for the two groups showed no statistically significant difference. However, a correlation between spiral ganglion cell density and the degree of cochlear inflammation, resulting primarily from infection, was statistically significant. The viability of the ganglion cells was verified by EABR recordings. There was good qualitative correlation between the shape of the EABR input-output function and cochlear pathology.

The results of this study indicate that long-term intracochlear electrical stimulation, using carefully controlled charge balanced biphasic pulses, does not adversely affect the spiral ganglion population or the cochlea in general.
5.1 Introduction.

Electrically activated neural prostheses are currently being investigated for a number of clinical applications. These include auditory (Simmons, 1966; Clark and Tong, 1982) and visual (Brindley and Lewin, 1968) prostheses, devices designed to control the paralyzed bladder in paraplegic patients (Nashold et al., 1977), electrical stimulation of the cerebellum to assist motor control in spasticity (Penn et al., 1978) and control of epilepsy (Oakley, 1977), and chronic stimulation of the phrenic nerve for diaphragm pacing in cases of chronic hypoventilation (Glenn et al., 1977).

These devices vary considerably in their electrode areas and the charge densities required to elicit neural activity (Brummer et al., 1983). An important requirement for the success of these devices is to ensure that the electrode does not adversely affect the adjacent biological environment during the charge injection process. One means of ensuring that charge injection is achieved by reversible electrochemical reactions is to use carefully controlled charge balanced biphasic pulses (Lilly, 1961; Brummer et al., 1977;
Maintaining Pt electrodes at charge densities below 300 uC cm\(^{-2}\) geom per phase ensures that irreversible faradaic reactions are avoided (Brummer et al., 1977; McHardy et al., 1980). However, stimulation within these limits in inorganic saline is known to result in Pt dissolution (McHardy et al., 1980). Recently, in a study in which protein was added to the saline electrolyte, Pt dissolution was found to be significantly reduced (Robblee et al., 1980). It is necessary to evaluate this inhibitory mechanism for in vivo stimulated electrodes. This is one objective of the present chapter.

A second important factor in determining the long-term effectiveness of a neural stimulator is the electrical stability of the stimulating electrodes. Such information can be obtained by monitoring the impedance of the stimulating electrodes during chronic stimulation. Subtle changes in electrode impedance has been interpreted as changes occurring at the electrode-tissue interface, or within the tissue immediately adjacent to the electrodes (Babb et al., 1977; Harrison and Dawson, 1977; Agnew et al., 1983). Long-term monitoring of electrode impedance in conjunction with an examination of the surface morphology of the stimulated electrodes is considered to be the most appropriate way of determining the effects of chronic electrical stimulation on the electrode (Johnson and Hench, 1977).
In addition, the design of current sourcing circuitry for a neural prosthesis depends - in part - on knowledge of the impedance of the stimulating electrodes. Loss of voltage compliance, due to high impedance electrodes, would result in an increase in the charge imbalance of the biphasic current pulse. These changes would result in an increased risk of tissue damage.

The present chapter describes a study designed to evaluate the effects of long-term intracochlear electrical stimulation on Pt electrodes using an electrode array and stimulus regime similar to that used in the Nucleus Limited Cochlear prosthesis. The impedance of these electrodes was monitored periodically during long-term electrical stimulation of the auditory nerve. These data were compared with similar data recorded from electrodes stimulated in inorganic saline. Following completion of the stimulation protocols the surface of these electrodes, together with unstimulated in vivo control electrodes, were examined for evidence of Pt dissolution using a SEM. The histopathological and physiological response to the chronic intracochlear electrical stimulation has been described in Chapter 4.

A brief review of the biochemical and biophysical properties of the electrode-tissue interface will precede a description of the methodology.
5.2 Objectives.

(i) to periodically monitor the ohmic and reactive components of the electrode impedance during chronic intracochlear electrical stimulation, and to compare these in vivo data with data obtained from similar electrodes stimulated in inorganic saline.

(ii) to examine the surface of in vivo and in vitro stimulated Pt electrodes for evidence of Pt corrosion, and to compare the surface of these electrodes with unstimulated in vivo control electrodes.

(iii) to examine the surface of the Silastic® insulator adjacent to the Pt electrodes of both the in vivo and in vitro scala tympani arrays.

5.3 Chronic Stimulating Electrodes: Biophysical and Biochemical Considerations.

Electrical stimulation of neural tissue involves the injection of current via electrons from a metal electrode to the adjacent electrolyte, where the charge carriers are ions. An understanding of the complex behaviour of the electrode-tissue interface and the
application of an appropriate electrical model, is required before changes in electrode impedance can be adequately interpreted.

When a metal electrode is placed in an electrolyte minute quantities of the metal dissolves into the electrolyte as positively charged metal ions. As a result of this process, the metal electrode becomes slightly negatively charged with respect to the electrolyte. The positively charged electrolyte layer adjacent to the metal electrode causes a reorganization of the charged species within the electrolyte, resulting in the production of two charged layers - the inner and outer Helmholtz layers (Fig. 5.1). The distribution of these layers depends on the properties of the metal electrode and the electrolyte.

The inner Helmholtz layer is formed by cations electrostatically attracted to the negatively charged electrode. These two charged layers constitute the double layer. Because the distance between these two layers is very small, the capacitance of the equivalent circuit is large. The outer Helmholtz layer is more diffuse than the inner layer due to the effects of thermal agitation, and has less influence on the electrical properties of the electrode-tissue interface.

In equilibrium conditions there is no nett current flow although the charge separation between the Helmholtz layers and the metal electrode produces a
potential across the interface (Fig. 5.1). Current flows when externally applied voltages modify this voltage gradient.

The impedance of the electrode-tissue interface is commonly modelled by a three element RC network consisting of a leaky capacitor and a series resistance (Weinman and Mahler, 1964; Pollak, 1974; Dymond, 1976; Fig. 5.2). This model has been used to satisfactorily describe a large number of electrode materials (including Pt) in biological fluids (Greatbatch and Chardack, 1968). This model must, however, be considered to be highly non-linear (Weinmann and Mahler, 1964; Dymond, 1976). Both the double layer capacitance, $C_{dl}$ and the faradaic impedance, $Z_f$, vary with current amplitude and stimulus time.

The response of the electrode voltage $V_e$ (Fig. 5.2) to a current pulse $i$, can be readily interpreted from the equivalent circuit. At the start of the pulse an abrupt rise in voltage is observed ($V_a$), and is equivalent to a voltage of $iR_a$. This purely ohmic response represents the instantaneous voltage developed across the electrodes as a result of the resistance of the electrolyte surrounding the electrode-tissue interface. An equivalent voltage drop ($iR_a$) is seen at the end of the current pulse. Changes in this ohmic component therefore reflect changes in the resistivity of the electrolyte adjacent to the electrodes. Throughout
Figure 5.1 Schematic diagram illustrating the inner and outer Helmholtz layers adjacent to a metal electrode.
the duration of the pulse the voltage across the electrode rises steadily as a result of the highly capacitive electrode-tissue interface. The shape of this portion of the electrode voltage is solely dependent on the reactive properties of the electrode-tissue interface ($C_d$ and $Z_f$).

The ohmic and reactive components of the electrode impedance can be derived from the electrode voltage and stimulus current waveforms. Monitoring both components can provide an important insight into electrochemical and biological changes occurring at or just beyond the electrode-tissue interface.

The suitability of various metal electrodes has been examined by an analysis of the electrode voltage waveform. To date however, Pt is the only electrode material which has been investigated to any extent under conditions appropriate for long-term stimulation (Brummer et al., 1983). The scope of possible charge injection processes during electrical stimulation with Pt electrodes, has been extensively investigated (Brummer et al., 1977; McHardy et al., 1980; Robblee et al., 1980). Initial charge injection is achieved by capacitive charging of the Helmholtz double layer. Such a charge injection process is electrochemically safe, as no new chemical species are formed. However, the limit of this charge injection process is limited to a charge density of about 5 uC.cm$^{-2}$ (Brummer et al., 1983). At
Figure 5.2 Equivalent circuit used to describe an electrode in electrolyte. $R_a$, the access resistance, models the ohmic resistance between the electrodes. The electrode-tissue interface is modeled by a capacitor $C_{dl}$ (the double layer capacitance) in parallel with a leaky resistor $Z_f$ (the faradaic impedance). The electrode voltage $V_e$ is shown for a current pulse $i$.

(from Black, 1978)
greater charge densities the equivalent of a dielectric breakdown occurs. The remaining charge injection is achieved via faradaic reactions.

For anodic first biphasic current pulses, oxide formation (OF) becomes the dominant charge injection process at charge densities in excess of 5 uC.cm\(^{-2}\) (Brummer et al., 1977). For Pt electrodes, oxygen evolution (OE) is observed at a charge density of about 300 uC.cm\(^{-2}\). Unlike OF, OE is an irreversible faradaic reaction resulting in the release of oxygen into the electrolyte via electrolysis of water. Such a reaction is highly undesirable, resulting in pH changes and the formation of new chemical products within the biological environment. By restricting the anodic pulse to a charge density of less than 300 uC.cm\(^{-2}\) and reversing the polarity of the current pulse via an equal charged cathodic pulse, Pt oxide formed in the anodic pulse is reduced and the electrode is returned to its original electrochemical state.

The response of a Pt electrode to a cathodic first biphasic current pulse produces a series of reversible and irreversible faradaic reactions similar to the previous example. Following initial charge injection via double layer charging, the charge injection process is dominated by oxide reduction (OR) and hydrogen atom plating (HP), both highly reversible electrochemical
reactions. Again, for charge densities in excess of approximately 300 uC.cm$^{-2}$ the cathodic first pulse begins to inject charge via irreversible $\text{H}_2$ evolution. Reversal of the current pulse prior to $\text{H}_2$ evolution will ensure that the electrode is returned to its original electrochemical state without the introduction of new, potentially toxic, chemical products.

The electrochemically safe injection of charge via Pt electrodes is therefore achieved by the highly reversible nature of surface Pt oxidation and reduction reactions, and hydrogen atom plating. Unlike the irreversible electrochemical reactions, these reactions are restricted to the electrode-tissue interface. However, the safe charge transfer process applies only to well charge balanced biphasic current pulses in which the reactions produced in the first phase are completely reversed in the second phase.

There remains, however, the possibility that small amounts of charge may be injected via secondary oxidation reactions such as oxidation of organic matter. Although these reactions have not been detected in vitro, they may be important in in vivo applications (Brummer et al., 1983). Furthermore, Pt dissolution does constitute a very minor form of charge injection even in in vivo environments (McHardy et al., 1980; Robblee et al., 1980). The potential toxicity of these reactions must be evaluated for each application.
5.4 Methods and Materials.

5.4.1 Electrodes.

The electrode arrays used in the present study have been described in Chapter 4. Briefly, the array consisted of two Pt ring-shaped electrodes wrapped around a Silastic® insulator. Each Pt electrode had an outside diameter of 0.6 mm and was 0.3 mm wide, providing a geometrical surface area of 0.56 mm². The electrode was manufactured from 25 μm thick Pt foil (99.95% Pt; Goodfellow Metals Ltd., UK), a strip of which was cut, wrapped around a mandrel and thermally welded two or three times. A multistranded, insulated Pt/Ir (90/10) leadwire (Leico Industries Inc., NY) was thermally welded to the inside surface of the electrode to provide contact with the stimulator. High purity materials were used in the manufacture of the electrode arrays as previous results have shown that metal impurities can result in pitting corrosion (Mears and Brown, 1941). In addition, the extent of cold metal working was kept to a minimum as there is evidence that this can also result in preferential corrosion (Mears and Brown, 1941; Rentler and Greene, 1975).

5.4.2 Implantation and stimulation protocols.

Both in vivo and in vitro stimulated electrodes were examined in the present study. The in vivo
electrodes were bilaterally implanted into the cochleae of 10 cats using a sterile surgical technique (Chapter 4). One cochlea of each animal served as a nonstimulated control while the other was stimulated continuously following a 10 day postoperative recovery period. The stimulation regime consisted of a biphasic constant current pulse of 200 μs per phase, presented at a rate of 500 pps. The electrodes were shorted between pulses to ensure that no residual charge could accumulate at the electrode-tissue interface. Previous electrochemical studies have shown a difference in anodal and cathodal electrode voltage curves following stimulation with these pulses (Greatbatch, 1967). These findings imply that there could be a residual post-stimulus charge remaining on the electrodes following the passage of the current pulse. Accumulated charge could have adverse effects on both the electrode and the adjacent tissue.

The current source stimulator was not capacitively coupled but charge asymmetries between phases were measured to be within 0.01-0.1% (Appendix I). Stimulus currents varied from 0.5-0.9 mA developing corresponding charge densities of 18-32 μC.cm⁻² geom. per phase. Table 5.1 summarizes the charge densities for all electrodes in the present study. Stimulus current and electrode voltage waveforms were monitored daily for both in vivo and in vitro stimulated
electrodes. In addition, electrode voltage waveforms were recorded at least twice weekly using the technique illustrated in Fig. 5.3. Electrode voltage waveforms for all in vivo electrodes were also recorded in vitro (in a normal saline filled Silastic® tube) prior to implantation and following explantation. In vivo control electrodes were not monitored. The access resistance \((R_a)\) and the electrode impedance \((Z_E)\) were derived from the electrode voltage and current waveforms.

The animals were electrically stimulated for periods ranging from 424-2029 hours with implantation times varying from 32-113 days. Each animal was sacrificed immediately following completion of its electrical stimulation programme and each electrode array was carefully removed from the cochlea. Care was taken to ensure that the electrodes were not contaminated by fixative as there is evidence that such contamination may result in corrosion artefact (McNamara and Williams, 1982). On removal, the electrode arrays were rinsed in distilled water, ultrasonically cleaned in distilled water then ethanol, and finally stored in ethanol until SEM examination.

\[ Z_E = \frac{V_p}{i} \]

\( Z_E \) We have defined electrode impedance \((Z_E)\) as the peak voltage of the electrode voltage waveform \((V_p)\) divided by the stimulus current \(i\).
Figure 5.3 Electrode voltage and current monitoring circuitry. The stimulus current from the current source stimulator was monitored by recording the voltage drop across a 1 kohm resistor placed in series with the stimulator ($V_1$-$V_2$). The electrode voltage waveform was monitored by recording the voltage across the electrode leads ($V_3$-$V_2$). In this example the electrode is modelled by a three element RC network. A Gould digital storage oscilloscope (OS4020) was used to monitor both waveforms. Recordings of the waveforms were obtained using a YEW 3022 X-Y plotter.
Eight in vitro electrode arrays were used in this investigation. Each array was placed in a small glass vial containing approximately 20 ml of pyrogen free, sterile, inorganic normal saline (Abbott Labs., Sydney). These vials were maintained at 37°C and the saline was changed daily. Four of the eight in vitro arrays (A,B,C and D) were stimulated using an identical regime to that used in the in vivo study. Charge densities varied from 18-36 \( \mu C cm^{-2} \) geom per phase, and stimulation times varied from 500-1500 hours (Table 5.1). The four remaining in vitro electrodes (E,F,G and H), were stimulated using a variety of stimulus regimes. Electrode voltage waveforms were not monitored for these four electrodes. One electrode array (in vitro E) was stimulated with an unbalanced biphasic stimulus regime for a period of 24 hours. The pulse width was 200 us per phase presented at a rate of 500 pps and a charge imbalance of approximately 62\%. A second array (in vitro F) was stimulated for 24 hours using a \(+10 \) mA monophasic pulse of 200 us duration, presented at a rate of 500 pps against a large Pt reference electrode. Finally, two electrodes (in vitro G and H) were stimulated with DC current at a current level of \( \pm 1.0 \) mA for a 12 hour period. The potential difference between these electrodes was 9.0 V. All in vitro electrodes were cleaned and stored according to the schedule outlined previously.
5.4.3 SEM analysis.

Just prior to SEM analysis each electrode array was trimmed and the intracochallear portion of the array, containing the two Pt ring electrodes, was adhered to a 10 mm diameter brass stub using high conductivity silver paint (Electrodag 915). The specimens were sputter coated with gold in order to provide a conductive coating over the Silastic® insulation. The electrodes were examined using a JEOL JSM-T200 SEM at an accelerating voltage of 25 kV and a working distance of 20 mm. The SEM was equipped with an Energy Dispersive X-ray Analysis (EDXA) facility. Both electrodes from each array were examined, and micrographs at low and high magnification were taken. Electrode surface features, including mechanical damage, surface pitting, stress corrosion cracking, intergranular corrosion and surface deposits were recorded. When examining these electrodes the investigator had no knowledge of whether the electrode was in vivo or in vitro, stimulated or a control array. The severity of Pt corrosion was arbitrarily graded from no corrosion (0 in Table 5.1) to extensive electrode corrosion (V in Table 5.1) for each electrode array. The Silastic® insulator was also examined for evidence of surface degradation.

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5.5 Results.

5.5.1 SEM results.

Table 5.1 contains a summary of the degree of corrosion for each electrode array in this study.

(a) In vivo electrodes.

Examination of the control in vivo electrodes revealed the presence of minor, localized pitting associated with surface score marks. This was due, in part, to the condition of the Pt foil used in the fabrication of these electrodes, and the manufacturing process itself, which included thermal welding and cold metal working. Fig. 5.4 illustrates the surface of an in vivo control electrode showing evidence of score marks and localized pitting in association with a generalized disruption of the metal surface as a result of a thermal weld. Score marks were associated with all in vivo electrodes examined. However, regions of surface pitting, if present, were generally restricted to weld zones. Examination of the surfaces of the electrodes stimulated in vivo revealed surface features very similar to those of the control group. Indeed, the variation in degree of corrosion for in vivo control and stimulated electrodes (Table 5.1) was not statistically significant (Wilcoxon Rank-Sum test). Fig. 5.5 is an example of the condition of the surface of the in vivo stimulated...
Table 5.1
Summary of stimulus regime and degree of corrosion for each electrode in this study.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Stimulus duration [h]</th>
<th>Charge density (μC·mm⁻² per phase)</th>
<th>Aggregate charge per phase [C]</th>
<th>Degree of corrosion</th>
<th>Type of corrosion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unworked PI foil control</td>
<td>0/11 sec</td>
<td>-</td>
<td>-</td>
<td>0/0</td>
<td>-</td>
</tr>
<tr>
<td>Balanced bipolar stimulus Electrodes</td>
<td>98R</td>
<td>2079</td>
<td>0.18</td>
<td>365</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>98L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>99R</td>
<td>1011</td>
<td>0.23</td>
<td>291</td>
<td>VI</td>
</tr>
<tr>
<td></td>
<td>99L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>G/C</td>
</tr>
<tr>
<td></td>
<td>100R</td>
<td>1115</td>
<td>0.21</td>
<td>241</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>100L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>D/A</td>
</tr>
<tr>
<td></td>
<td>107R</td>
<td>1128</td>
<td>0.18</td>
<td>311</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>107L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>108R</td>
<td>1189</td>
<td>0.22</td>
<td>305</td>
<td>VI</td>
</tr>
<tr>
<td></td>
<td>108L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>G/C</td>
</tr>
<tr>
<td></td>
<td>109R</td>
<td>1529</td>
<td>0.21</td>
<td>220</td>
<td>VI</td>
</tr>
<tr>
<td></td>
<td>109L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>112R</td>
<td>1514</td>
<td>0.21</td>
<td>327</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>112L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>117R</td>
<td>529</td>
<td>0.21</td>
<td>116</td>
<td>SP</td>
</tr>
<tr>
<td></td>
<td>117L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>132R</td>
<td>558</td>
<td>0.18</td>
<td>102</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>132L</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>III</td>
</tr>
<tr>
<td>In vivo electrodes</td>
<td>A</td>
<td>600</td>
<td>0.21</td>
<td>108</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>500</td>
<td>0.36</td>
<td>180</td>
<td>IV/N</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1500</td>
<td>0.18</td>
<td>270</td>
<td>IV/N</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1000</td>
<td>0.18</td>
<td>180</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>24</td>
<td>0.72</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>24</td>
<td>3.57</td>
<td>460</td>
<td>N/D</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>12</td>
<td>-</td>
<td>+43</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>12</td>
<td>-</td>
<td>+43</td>
<td>V</td>
</tr>
</tbody>
</table>

Key: D. No corrosion present; I, possible slight corrosion; II, localized minor corrosion; III, localized moderate corrosion; IV, widespread corrosion; V, extensive and extensive corrosion; D, corrosive deposit; C, electrode surface covered with Siemens phosphorus layer; N/A, not available for examination; SP, surface pitting corrosion; DCC, dense corrosion cracking; IG, intergranular corrosion; +, data determined from phase with largest current amplitude.

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electrodes. In addition, there was no correlation between the duration of electrical stimulation and the degree of corrosion (Fig. 5.6), or charge density (or total aggregate charge injected) and the degree of corrosion (Fig. 5.7). Two in vivo electrode arrays (112R and 117R) were covered with Silastic® and fibrous tissue, and were therefore not included in the present analysis. Partial fibrous tissue cover of a number of other in vivo electrodes was observed (e.g. Fig. 5.12). The fibrous tissue cover was observed equally over in vivo stimulated and control electrodes, and was restricted to the Pt surface. One in vivo stimulated electrode (107R) showed evidence of more widespread surface pitting corrosion restricted to one electrode of the pair. The reason this electrode had widespread pitting is unclear, its stimulus regime was not extreme in terms of charge density or stimulus duration, and the electrode evoked a mild tissue reaction indicating minimal inflammation. It is possible that the surface examined included an extended weld zone.

(b) In vitro electrodes.

In vitro electrodes A, B, C and D were stimulated using an identical stimulus regime to that used for the in vivo electrodes. However, the condition of the surfaces of these electrodes varied significantly. Arrays A and D, stimulated at relatively low charge
densities and for only moderate durations (Table 5.1), had Pt surfaces with little evidence of corrosion and were comparable with in vivo electrode surfaces. Significantly, electrodes B and C showed evidence of widespread surface pitting, stress corrosion cracking and intergranular corrosion (Fig. 5.8). In each case one electrode of the pair showed extensive corrosion whilst the second electrode exhibited a less marked degree of corrosion. This probably reflects different electrochemical reactions occurring at each electrode due to their opposite polarity leading phases.

Electrode array in vitro E was stimulated with a poorly balanced biphasic stimulus for a 24 hour period. The electrodes exhibited Pt surfaces similar to those exhibited by the in vivo electrodes. In vitro F, stimulated with a +10 mA (peak), 200 us monophasic stimulus, showed the presence of a deposit covering much of the Pt surface. EDXA analysis indicated that the deposit was predominantly platinum. This electrode did not show evidence of widespread pitting corrosion. Finally, in vitro electrodes G and H, stimulated with DC current for a period of 12 hours, exhibited evidence of severe Pt corrosion. In both cases a significant proportion of the electrode had undergone dissolution. It would appear that the corrosive activity was concentrated at the edge of the electrode in the form of intergranular attack, while certain zones away from the

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failure exhibited stress influenced corrosion highlighted by parallel fractures (Fig. 5.9)

There was no indication of degradation of the Silastic® insulator in either in vivo stimulated or control electrodes, or in vitro electrodes stimulated with a balanced biphasic stimulus regime. Silastic® degradation was observed on one in vitro electrode array stimulated using a DC stimulus current.

5.5.2 Electrode impedance.

Representative electrode impedance ($Z_\text{e}$) and access resistance ($R_\text{a}$) data, plotted as a function of implantation time, are illustrated in Figs. 5.10 and 5.11. Both in vivo and in vitro stimulated electrodes are illustrated in these examples.

Figure 5.10 shows the impedance measured from four chronically stimulated scala tympani electrode arrays. These data illustrate the range of individual variability observed among the in vivo electrodes. Although these electrodes were stimulated at different current levels (101R, 0.6 mA; 107R, 0.5 mA; 108R, 0.9 mA and 117R, 0.5 mA), both $R_\text{a}$ and $Z_\text{e}$ varies linearly with current amplitude at a rate of -350 ohm/mA (for the current range 0.1-1.0 mA). Such small linear changes, therefore have little effect on the measured impedance.

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Figure 5.4 SEM micrograph of in vivo control electrode 107L(1). Surface features include score marks associated with the manufacture of the electrode and regions of localized pitting (arrowed) within a weld zone (W). The weld has resulted in a general disruption of the metal surface. A deposit probably of organic origin, is also apparent. Bar: 100um.

Figure 5.5 SEM micrograph of in vivo stimulated electrode 134R(1). This electrode was stimulated for 568 h at a charge density of 18 uC.cm⁻² geom. per phase. Although score marks were present, there was little evidence of pitting corrosion. Pt, platinum electrode; S, Silastic®. Bar: 100um.
Figure 5.6 Surface of in vivo electrode 98R(1). This electrode was stimulated for 2029 h at a charge density of 18 uC. cm$^{-2}$ geom. per phase. The general condition of the Pt surface was excellent, although a local region of pitting is apparent (arrowed). Erythrocytes are also present. Bar: 100µm.

Figure 5.7 Surface of in vivo electrode 108R(1) after 1189 h of stimulation, having injected 385C of charge (per phase) at a charge density of 32 uC.cm$^{-2}$ geom. per phase. This was the highest aggregate charge and charge density delivered in the in vivo series. Although manufacturing marks were apparent, pitting corrosion was not present.

Bar: 100µm.
Figure 5.8 Surface of in vitro electrode B(II) following 500 h of stimulation in inorganic saline at a charge density of 36 uC.cm$^{-2}$ geom per phase. Extensive surface pitting in the form of intergranular corrosion is apparent. Bar: 100um.

Figure 5.9 Edge of in vitro electrode H, showing evidence of severe intergranular Pt corrosion (arrows) following 12 h of DC (cathodal) stimulation. Stress induced parallel fractures are evident on the electrode surface. Bar: 10 um.
Although these data illustrate large individual variations, they do exhibit a number of similarities. First, in vitro $R_a$ and $Z_E$ measured just prior to implantation and just following explantation (shown in these examples as filled symbols), are similar for a given electrode, and somewhat less than the in vivo measured values (e.g. 117R, Fig. 5.10). As these in vitro data were measured in a normal saline filled tube of approximately the same internal diameter as the basal scala tympani of the cat, these findings suggest that perilymph has a greater resistivity than normal saline. Further, the similarity in these in vitro data suggest that the changes in electrical characteristics observed in vivo, reflect changes occurring within the adjacent biological environment rather than the electrode surface. The apparent dynamic properties of the tissue interface are further emphasized when the in vivo monitored $R_a$ and $Z_E$ are compared with the highly stable values monitored during chronic in vitro stimulation (e.g. in vitro $R$, Fig. 5.11). Second, in vivo $R_a$ and $Z_E$ generally exhibited a gradual increase during the first 12-30 days following implantation within the scala tympani. Following this period, both $R_a$ and $Z_E$ remained relatively constant except, however, for some rather significant short-term fluctuations. These short-term variations were observed for the majority of the in vivo data. The long-term changes observed in $Z_E$ generally
reflect the purely resistive changes of $R_a$. It would appear, therefore, that these long-term changes reflect a gradual increase in the resistive pathways between the two electrodes. Finally, the effect of chronic electrical stimulation (indicated in these figures by full lines between data points), does not appear to have any significant effect on the behaviour of $R_a$ and $Z_F$ measured in vivo (e.g. 107R, Fig. 5.10).

Figure 5.11 shows the impedance measurements of three chronically stimulated in vitro electrodes. These data are compared with the most stable in vivo impedances recorded (134R). In contrast with in vivo results, pre- and post-stimulus in vitro results (measured in a narrow diameter Silastic® tube) were similar to the impedance values measured during stimulation (measured in a 100 ml saline filled beaker). These in vitro impedance measurements were very stable and $R_a$ and $Z_F$ values were significantly less than the in vivo results. These in vitro data imply a stable electrical environment associated with the electrodes.

Like the in vitro data, $R_a$ and $Z_F$ measured from electrode 134R reflects a stable electrode-tissue interface and proximal biological environment. This observation is supported by both histopathological data (Chapter 4) and SEM observations, as it was one of only two electrodes that did not evoke a fibrous tissue reaction following chronic scala tympani implantation.
Figure 5.10 Electrode impedance (■) and access resistance (▲) in kohms as a function of implantation time for four chronically stimulated in vivo bipolar electrodes. Solid symbols indicate in vitro measurements recorded prior to implantation and after explantation. Dashed lines indicate recordings made prior to the start of continuous electrical stimulation.
**Figure 5.11** Electrode impedance and access resistance in kohms as a function of implantation time for one in vivo (134R) and three in vitro chronically stimulated electrodes. Note the stability of both $R_a$ and $Z_r$ in these four examples, compared with the examples illustrated in the previous figure.
Moreover, SEM analysis of the electrode surfaces showed no evidence of fibrous tissue (or any other) deposits (Fig. 5.5).

5.6 Discussion.

Implantable prostheses are subjected to a severe corrosive environment. Indeed, the majority of metals corrode when implanted in tissue (McFadden, 1969; Brown and Merritt, 1980; Clark and Williams, 1983; Loeb, 1983). In addition, devices such as neural prostheses are subjected to additional corrosive stresses as a result of the charge injection process (Lilly, 1961; McHardy et al., 1980; Brummer et al., 1983; Loeb, 1983).

The adverse effects of stimulating Pt electrodes with monophasic or DC current is well known (Loucks et al., 1959; Black and Hannaker, 1979; Brown and Merritt, 1980), and has been illustrated qualitatively in the present study.

Comparison of in vivo stimulated and control electrodes, in the present study, revealed no significant difference in their electrode surface characteristics although the stimulated electrodes had been activated continuously for periods of up to 2000 hours at charge densities of up to 32 uC.cm\(^{-2}\) geom. per phase. This finding suggests that long-term in vivo stimulation using charged balanced biphasic pulses does not affect the corrosion properties of the stimulated electrodes. This
observation was supported by the histopathological results which indicated that the extent and density of the fibrous tissue capsule associated with the electrode array was not related to the degree of electrical stimulation (Chapter 4). Previous studies have indicated that there is a correlation between the extent of fibrous tissue response and the degree of tissue irritation due, for example, to the injection of excessive charge into the biological environment, or to toxic effects of metal dissolution (McFadden, 1969; Dymond et al., 1970; Homsy, 1970; Bernstein et al., 1977).

In contrast to the in vivo results, the in vitro electrodes stimulated chronically using a charge balanced biphasic stimulus, showed marked Pt corrosion associated with high charge density (36 uC.cm\(^{-2}\) geom. per phase) or aggregate charge (270 C per phase; Table 5.1; Fig. 5.8). Unlike the in vivo electrodes, these electrodes showed evidence of intergranular corrosion. Significantly, these electrodes were stimulated at similar charge densities and for similar periods to a number of in vivo electrodes. The qualitative results obtained in the present study are consistent with a number of electrochemical studies that have shown Pt dissolution in inorganic saline to be dependent on the aggregate charge injected and the charge density developed at the electrode surface (Black and Hannaker,
1979; McHardy et al., 1980). Significantly, the addition of small quantities of protein markedly reduces the Pt dissolution rate (Robblee et al., 1980). It would, therefore, appear that proteins play a significant role in the inhibition of platinum dissolution. The results of the present study indicate an inhibitory effect in vivo. It seems likely that the presence of proteins may contribute to this effect.

The present findings are consistent with a recent study in which trace analysis of Pt was determined in tissue adjacent to cortical surface electrodes. The rate of Pt dissolution was shown to gradually decrease during the 36 hour stimulus period and suggests a progressive inhibition of Pt dissolution during the stimulus period (Robblee et al., 1983). As these electrodes were implanted one month prior to the acute stimulation period, it would appear that the activation of the inhibitory mechanism is dependent on the injection of charge in the presence of protein. These data are consistent with in vitro results obtained following the addition of protein to an inorganic electrolyte (Robblee et al., 1980).

The establishment of localized potential differences across grain boundaries, resulting in electrochemical corrosion of metal surfaces, can be due, as previously discussed, to a number of factors including metal impurities, differing thermal treatment of the
metal surface, and the variation and extent of cold metal working (Mears and Brown, 1941; Rentler and Greene, 1975). In the present study, small regions of localized surface pitting were occasionally observed on both stimulated and control in vivo electrodes (Table 5.1; Figs. 5.4 & 5.6), with the majority of pitting being associated with weld zones (Fig. 5.4). Although the extent of Pt corrosion from these effects was not significant, this finding does serve to illustrate the importance of restricting the extent of cold metal working, differential thermal treatment and the possibility of metallic contamination during the manufacture of the implantable prosthesis†. Platinum ring electrodes now used in the multi-channel Cochlear prosthesis manufactured by Nucleus Ltd., are produced directly from commercially available, high purity (99.95%) platinum tubing, thereby minimizing the degree of cold metal working and thermal welding, and reducing the sources of metallic contamination. SEM analysis of these electrodes has indicated a significant improvement in the condition of the electrode surface (Fig. 5.12).

† Although there are a number of possible sources of metallic contamination during the manufacture of an electrode array, the most probable source in this case is the deposit of small amounts of the tungsten welder onto the Pt foil during the thermal welding process.
Figure 5.12  SEM micrograph of a Pt electrode manufactured from Pt (99.95%) tubing. Electrodes manufactured this way show significantly less mechanically induced surface damage compared with electrodes manufactured from Pt foil. Further, these electrodes are subjected to significantly less cold metal working and thermal welding. Pt, platinum electrode; S, Silastic®. Bar: 100um.
Although the present results have shown that there is minimal visible corrosion of in vivo stimulated Pt electrodes operating close to the upper stimulus level for the multi-channel Cochlear prosthesis, there are many applications in which neural prostheses operate at significantly greater charge densities (Brummer et al., 1983). There is some evidence to suggest that significant dissolution in vivo stimulated electrodes occurs at charge densities as low as 200 uC.cm\(^{-2}\) geom. per phase for Pt/Ir (70/30) electrodes (Agnew et al., 1983). The use of activated iridium electrodes may be more suitable for applications requiring high charge densities (Brummer et al., 1983; Robblee et al., 1983).

In contrast to the stable \(R_a\) and \(Z_E\) data recorded for all in vitro stimulated electrodes, the in vivo data exhibited large variations in both \(R_a\) and \(Z_E\) among electrodes. For example, electrode 134R (Fig. 5.11) showed minimal changes in both \(R_a\) and \(Z_E\) throughout the implantation period of 44 days. These results infer a stable tissue environment adjacent to the electrode pair. Subsequent histological examination of the cochlea and SEM analysis of the electrodes confirmed that this electrode array did not evoke a fibrous tissue capsule (Figs. 4.5 & 5.5). This electrode was therefore in an environment quite similar to the in vitro electrodes, i.e. bathed in an electrolyte (in this case perilymph) without any local fibrous tissue activity.
In contrast, electrode 117R exhibited the highest in vivo $R_a$ and $Z_E$ values measured in this series (Fig. 5.10). Histological examination of the cochlea implanted with this electrode revealed a compact fibrous tissue capsule which completely encapsulated the electrode array. Furthermore, SEM analysis of the electrode surface revealed an extensive covering of fibrous tissue (and possibly Silastic®) over both electrodes (Table 5.1). In this example, the fibrous tissue covering the electrode was so extensive that inspection of the Pt surface was not possible. Considering the vigorous cleaning process that these electrodes are subjected to prior to SEM evaluation, the fibrous tissue would appear to be firmly attached to the Pt surface. Presumably, close attachment of fibrous tissue to the electrode surface would affect the electrical characteristics of the electrode. An example of fibrous tissue partially covering a Pt electrode surface is shown in Fig. 5.13. Extensive covering of fibrous tissue, as observed with electrode 117R, did not frequently occur.

Although in vivo electrodes 101R, 107R, 108R and 117R were all associated with a general fibrous tissue reaction within the basal turn scala tympani, the increase observed in $R_a$ and $Z_E$ appeared to be independent of this tissue reaction. The most obvious association between fibrous tissue and electrode impedance appeared
to be the density and continuity of the fibrous tissue capsule enveloping the electrode array. For example, cochleas 117R and, to a lesser extent, 101R, showed near complete fibrous tissue capsules surrounding the electrode array. Moreover, these tissue capsules were several cells thick. Both electrodes exhibited electrode impedances that increased, over approximately the first 20 days following implantation, to relatively high values. This observation is consistent with the time course required for the growth of a tissue capsule. In contrast, histological examination of cochleas 107R and 108R revealed fine tissue capsules of only one or two cells thick (e.g., 108R, Fig. 4.7). Furthermore, these tissue capsules did not completely envelope the electrode array.

These observations suggest that the density and continuity of the fibrous tissue capsule formed around a scala tympani electrode array, contributes to the electrical characteristics of the electrode. Moreover, these changes in electrode impedance are basically resistive, indicating that the fibrous tissue reaction contributes to increases in $R_a$, the access resistance, while seemingly having little effect on the reactive components of $Z_E$. Similar observations have been made by others (Agnew et al., 1981).
The marked short-term fluctuations of both $R_a$ and $E_z$ observed in the present study (e.g. 101R & 117R, Fig. 5.10) have also been reported by other investigators using both scala tympani electrodes (Byers et al., 1981) and electrodes implanted in other sites within the body (Sabb et al., 1977; Agnew et al., 1981). Although the origin of these fluctuations is unclear, they appear dependent on the extracellular environment. This fluctuating behaviour was only observed among in vivo stimulated electrodes, and therefore reflects the dynamic properties of the biological environment.

One possible contributor to these short-term fluctuations in electrode impedance may be the effects of protein on the electrode surface. Protein is known, from in vitro electrochemical studies, to form a surface film that retards the movement of Pt ions from the electrode surface (Robblee et al., 1980). This process is dynamic, as the Pt inhibition mechanism requires the injection of a finite amount of charge before it becomes established (Robblee et al., 1980; Robblee et al., 1983). Moreover, this inhibitory mechanism is only observed as long as there is a constant supply of protein. If protein is removed from the electrolyte Pt dissolution again increases.

Although not commonly observed on electrodes in the present study (probably due to the rigorous cleaning schedule each electrode underwent prior to SEM...
examination), a thin, presumably proteinaceous film covering a Pt electrode is shown in Fig. 5.14. In this example the scala tympani electrode array had been implanted in a patient for a period of 3.8 years. Erythrocytes, some of which can still be seen on the electrode, have created small holes in this surface film. Similar films have been observed by others following chronic in vivo stimulation (Johnson and Hench, 1977).

The presence of these protein films would presumably alter the electrical properties of the electrode-tissue interface. Furthermore, the maintenance of this film could be quite a dynamic process as denatured proteins would be continually replaced. Interestingly, in the present study, short-term fluctuations in both $R_a$ and $Z_E$ generally occurred some time after commencement of electrical stimulation (e.g. 117R, Fig. 5.10). It may be that the stimulus induced protein inhibition of Pt corrosion, may also contribute to the short-term fluctuations observed in electrode impedance. Clearly, these complex changes in electrode impedance are a function of the dynamic properties of the in vivo environment.

The appearance of a proteinaceous exudate in a number of chronically stimulated cochleas (see Chapter 4), would probably be associated with an increased protein concentration in the perilymph. However, there
Figure 5.13  Fibrous tissue (f) attached to the surface of in vivo electrode 99R. This electrode was stimulated for 1011 hours at a charge density of 28 uC.cm$^{-2}$ geom. per phase. The Pt surface showed no evidence of corrosion.  

Bar: 10um.

Figure 5.14  A thin proteinaceous film can be seen covering a large area of a Pt electrode surface. A number of erythrocytes are also attached to the electrode surface, although many have been removed leaving holes of approx. 8 um diameter in this film.  

Bar: 10um.
was no correlation between electrode impedance and the presence of an exudate. Presumably then, if the formation of a protein film over the Pt electrodes does contribute to these fluctuations in electrode impedance, increasing the protein concentration within the perilymph does not appear to enhance this effect. This observation has some parallel with protein inhibition of Pt dissolution, which has been shown to be independent of protein concentration (Robblee et al., 1980).

The short-term fluctuations observed in the present study highlights the importance of using current source rather than voltage source stimulators for neural prostheses. Since the excitation of neural tissue is basically a charge dependent process (see Chapter 2), current injection into neural tissue must be controllable. Unlike current source stimulators (operating within the limits of voltage compliance), current regulation cannot be controlled using voltage source stimulators in environments in which there are large fluctuations in electrode impedance.

Finally, the observation that there was extensive Pt dissolution occurring on the surface of stimulated in vitro electrodes B and C (Table 5.1), while their respective $R_a$ and $Z_E$ data remained stable (Fig. 5.11), raises questions about the use of electrode impedance data as an indicator of the condition of the surface of the metal electrode. Johnson and Hench
(1977) have made similar observations following chronic stimulation of gold electrodes. They reported stable electrode impedances while SEM analysis of the electrodes revealed extensive pitting corrosion. These observations indicate that the use of $C_d$ and $Z_r$ (Fig. 5.2) as indicators of the condition and effective surface area of metal electrodes, as suggested by some authors (e.g. Agnew et al., 1982), may be misleading. Although $R_a$, the ohmic component of electrode impedance, appears to be a good indicator of the activity of the tissue environment proximal to the electrodes, the condition of the surface of these electrodes is best determined using scanning electron microscopy.

5.7 **Summary.**

Resistive and reactive components of electrode impedance were monitored for Pt bipolar scala tympani electrode arrays during chronic electrical stimulation using charge balanced biphasic current pulses at charge densities in the range 18-32 uC.cm$^{-2}$ geom. per phase. These data were compared with similar data obtained from electrodes stimulated in inorganic normal saline. In vivo stimulated electrodes exhibited large individual variations in electrode impedance. A number of electrodes showed little change in impedance throughout the implantation period. Other electrodes showed threefold increases in impedance over approximately the
first 20 days post implantation, after which the impedance remained relatively constant. These essentially resistive changes have been attributed to the growth of a fibrous tissue capsule enveloping the implanted electrode array. The more extensive the tissue capsule, the greater the resistive component of the electrode impedance.

Many of the in vivo stimulated electrodes exhibited fairly large short-term fluctuations in electrode impedance. The origin of these variations is unclear, although it is suggested that a protein film, thought to be covering these electrodes, may contribute to this effect. These short-term fluctuations in impedance were only observed in in vivo stimulated electrodes. These findings emphasize the need for neural prostheses to use current source rather than voltage source stimulators to ensure controlled injection of charge into the biological environment.

In vitro stimulated electrodes exhibited stable electrode impedances, however subsequent SEM analysis of the surface of two of these electrodes revealed extensive Pt pitting corrosion. This suggests that electrode impedance is not a reliable indicator of the condition or the surface area of electrodes.

SEM analysis of the in vitro electrodes revealed evidence of Pt corrosion for electrodes stimulated at a high charge density (36 uC.cm\(^{-2}\) geom. per phase) or
aggregate charge injection (270°C). Significantly, in vivo electrodes stimulated with similar stimulus regimes showed no evidence of stimulus induced corrosion. Indeed, their surfaces were similar to in vivo control (unstimulated) electrodes. This finding indicates that chronic in vivo stimulation using this stimulus regime does not place undue electrochemical stress on the Pt electrodes.

In vitro electrochemical studies have demonstrated that protein plays a significant role in the inhibition of Pt dissolution. The present study has demonstrated an inhibitory effect in vivo. This may be due to the presence of protein.
CHAPTER SIX

ACUTE INTRACOCHLEAR ELECTRICAL STIMULATION USING HIGH STIMULUS INTENSITIES AND PULSE RATES: PHYSIOLOGICAL AND ULTRASTRUCTURAL RESULTS.

6.1 Introduction.

Although non-damaging electrical stimulus parameters have been described for chronic intracochlear electrical stimulation (Chapter 4), maximum safe stimulus levels have yet to be defined. Moreover, the significance of stimulus parameters other than charge density, on maximum safe stimulus levels, is not well understood.

Charge density per phase (μC.cm⁻² geom. per phase) appears to closely correlate with the degree of neural damage. Provided that charge density is maintained below approximately 40-50 μC.cm⁻² geom. per phase, for short duration charge balanced biphasic current pulses and Pt electrodes, there is little evidence of neural damage. Above this value, the extent of neural damage appears to increase with charge density (Pudenz et al., 1975; Agnew et al., 1975; Brown et al., 1977; Pudenz et al., 1977; Yuen et al., 1981; Walsh and Leake-Jones, 1982; Agnew et al., 1983). These findings are consistent for relatively large surface area electrodes such as those used in the Cochlear prosthesis.
(i.e. 0.5-1.0 mm²). However, significantly higher non-damaging charge densities have been reported when stimulating with microelectrodes (areas in the order of \(10^{-4}-10^{-6}\) mm²). For example, stimulation with Pt/Ir intracortical microelectrodes at charge densities as high as 300 \(\mu\text{C.cm}^{-2}\) geom. per phase, failed to produce neural damage during 24 hours of continuous electrical stimulation (Agnew et al., 1982). In contrast, stimulating with larger surface area electrodes, neural degeneration has been observed following acute stimulation using a similar stimulus regime at charge densities as low as 40 \(\mu\text{C.cm}^{-2}\) geom. per phase (Yuen et al., 1981). These conflicting results suggest that stimulus parameters other than charge density must also be considered when evaluating maximum biologically safe stimulus levels.

Since these neural damage levels are significantly less than the limits imposed by electrochemical considerations (Brummer et al., 1977), neural damage mechanisms - at least for stimulus levels operating just above these biologically safe stimulus limits - would appear to be due to the effects of electrical stimulation per se, rather than as a result of adverse electrochemical reactions.

The present chapter describes a study designed to evaluate the physiological and histopathological response of the auditory nerve to acute periods of
intracochlear electrical stimulation at high stimulus intensities and stimulus rates. An understanding of the response of the auditory nerve to these extreme stimulus parameters should provide an insight into neural damage mechanisms associated with electrical stimulation, and help define maximum safe stimulus levels for this device. Knowledge of maximum safe stimulus levels is a prerequisite to the design and evaluation of new innovative speech processing strategies.

In this study, EABRs were monitored prior, during and periodically following the completion of periods of acute stimulation at defined stimulus intensities, rates and durations. Because high stimulus intensities were used, animals were under general anaesthesia for the duration of the experiment (typically 48-72 hours). As long-term monitoring of the auditory nerve was required, the EABR was considered to be the most appropriate monitoring technique. Single unit recording of auditory nerve fibres was not considered practicable due to the difficulty in recording from individual units for long periods of time (i.e. hours). Moreover, the presence of a large stimulus artefact, especially at high stimulus intensities, made gross recording the auditory nerve (N1b), difficult (although this procedure was used in a number of experiments in the present study). Latter waves of the EABR (e.g. wave IV), are far less susceptible to
artefact contamination. Furthermore, these waves reflect both amplitude and latency changes occurring at the level of the auditory nerve (Chapter 2), they are robust, and can be recorded over long periods of time.

6.2 Objectives.

(i) to use EABR and electrically evoked N1 responses to monitor stimulus induced changes in the auditory nerve during periods of acute intracochlear electrical stimulation at defined stimulus intensities and stimulus rates.

(ii) to monitor recovery of the auditory nerve, using these recording techniques, following completion of periods of acute intracochlear electrical stimulation, and to compare these stimulus induced changes with responses obtained from implanted, contralateral control cochleas.
(iii) to use the transmission electron microscope to examine the auditory nerve at the ultrastructural level, following both temporary and permanent stimulus induced changes in sensitivity of the evoked response and to compare these ultrastructural details with suitable control cochleae.

(iv) to use these physiological and ultrastructural data to investigate the pathophysiological mechanisms underlying both temporary and permanent stimulus induced changes in the electrically stimulated auditory nerve.

6.3 Methods and Materials.

This study was conducted according to the National Health and Medical Research Council guidelines for animal experimentation.

6.3.1 Preoperative preparation.

A total of 15 normal hearing and neomycin deafened adult cats were used in the present study. Each animal had otoscopically normal tympanic membranes. Normal hearing animals had ABR thresholds to 0.1 ms acoustic clicks of less than 37 dB peak equivalent
sound pressure level (pe SPL, re 20 uPa; ABR recording techniques have been described in section 4.3.4). Deafened animals were systemically administered with neomycin sulphate (Mycifradin®, Upjohn), using a dose of 50 mg/kg i.m. over a period of 10 consecutive days. A number of animals were briefly sedated with halothane (Fluothane®, ICI) prior to the intramuscular injection of neomycin. Although there was considerable individual variation in response to the deafening process among these animals, by a period of 90 days following the administration of the drug all animals failed to evoke an ABR to an acoustic click presented at a stimulus level of 87 dB pe SPL (Shepherd and Clark, 1985). These profoundly deafened animals were used in the present study following a minimum period of 120 days after administration of the drug.

6.3.2 Electrodes.

The scala tympani electrodes used in the present study were manufactured using the same materials and techniques as described in Chapters 4 & 5. The electrode arrays used in this study typically contained four to six Pt ring electrodes. These electrodes were 0.3 mm wide and had a diameter of 0.4 mm, providing an electrode surface area of approximately 0.37 mm². Each electrode was connected to a leadwire in a manner similar to that described in Chapter 4.
6.3.3 Surgery.

The animals, whose weights were in the range 2.0-4.4 kg, were anaesthetized with an i.p. injection of ketamine hydrochloride (18 mg/kg; Ketalar®) and xylazine (3.8 mg/kg; Rompun®). Anaesthesia was maintained for the duration of the experiment with sodium pentobarbitone (Nembutal®). Initially, the animal was given a dose of 30 mg/kg i.p. over the first four hours following induction. Thereafter, the pedal reflex was used to periodically monitor the state of anaesthesia and sodium pentobarbitone was administered i.p. as required. Following induction, each animal was given 250 ug of atropine sulphate i.m. (David Bull Labs.). Additional 250 ug quantities were administered every 12 hours for the duration of the experiment. Atropine reduces salivary secretion and therefore reduces the risk of a respiratory occlusion. This drug also reduces the depressive effects of barbiturate anaesthetics on heart rate (Sellick and Russell, 1979). In addition, 250 mg each of the antibiotics Cloxacillin and Ampicillin sodium (CSL), were dissolved in sterile water and administered i.p. every 24 hours. Finally, 60 ml of a 5% dextrose in normal saline solution (Abbott Labs.) was given s.c. every 24 hours.
The animal was shaved and the surgical sites cleaned with an antiseptic solution. The animal was then placed on a heated operating table where all surgery was performed. The trachea was cannulated to reduce respiratory obstruction. The patency of the tracheal cannula was monitored throughout the experimental procedure and the trachea was gently aspirated if the animal had difficulty breathing. Following this procedure, the animal was placed in a Trent Wells stereotaxic frame and securely mounted to it through clamps to the mouth and orbital margins of the eyes, and through ear bars placed in the external auditory meatus. The skull was exposed and the rostral portion of the animal's head was further secured by fixing a bar to the frontal sinus with stainless steel screws and attaching this bar to the stereotaxic frame. This procedure allowed the ear bars and the side frame of the stereotaxic apparatus to be removed, freeing the caudal region of the skull for surgery.

The auditory bulla and round window were exposed as described in Chapter 4. Just prior to insertion of the electrode, a small amount of wax was melted onto the bone overlying the superior-rostral region of the exposed bulla. The round window was incised with a 25 G needle. The inferior-laterally based incision was generally restricted to twice the diameter of the 25 G needle. This procedure frequently resulted in a small 6-8
amount of perilymph leakage, bleeding was only observed occasionally. A rostral approach to the round window would ensure an adequate view along the basal turn scala tympani. The electrode was inserted for a distance of up to 6 mm along the scala. The leadwire close to the cochlea was fixed to the wax and more distal leadwire was fixed to the wound using cyanoacrylate. The exposure overlying the the bulla was then sealed in wax which provided further fixation of the electrode system. Both cochleas of each animal were implanted, one side being electrically stimulated while the other served as a control. Finally, a small hole was drilled at the vertex to the level of the dura. A stainless steel screw was placed in this hole and served as the vertex recording electrode for EABRs.

Following completion of the surgery, the animal was placed onto a DC powered heating blanket (Epil, 240A). A rectal thermometer was used to monitor the animal's body temperature and regulate the heating blanket. The animal was maintained at 37-39 °C.

6.3.4 EABR and N1g stimulus and recording techniques

EABR stimulus and recording techniques have been discussed previously (4.3.4), and block diagrams are illustrated in Appendix I. In the present experimental series EABRs were recorded differentially using the stainless steel screw as the vertex electrode and
subcutaneous needle electrodes at the neck and thorax (Vertex, +ve; neck, -ve; thorax, ground). This bilaterally symmetrical recording configuration enabled recordings to be made from either ipsi- or contralateral cochleae without reconfiguring the recording electrodes. Furthermore, this recording configuration emphasized the latter waves of the EABR (i.e. waves III & IV) at the expense of wave I. Wave IV (2.6 - 3.2 ms) of the EABR was routinely monitored as it was the lowest threshold wave in the response, and was never corrupted by stimulus artefact. By placing the vertex recording electrode under the skull, the amplitude of the EABRs were increased compared with electrodes placed subcutaneously. This increase in signal to noise allowed fewer responses to be required for averaging. In the present study 100 responses were averaged for each EABR recording. Current pulses evoking the EABR were routinely presented at 33 pps.

In two experiments the N1P was monitored so as to compare stimulus induced changes recorded at the level of the auditory nerve with changes recorded within the generators of wave IV. In these experiments a craniotomy was performed by opening the posterior fossa. The dura was cut and the cerebellum overlying the auditory nerve and cochlear nucleus was aspirated. A small Pt/Ir (90/10) ball electrode was placed on the VIIIth nerve just as it exited from the internal auditory
meatus. This procedure was performed under magnification. The location of the recording electrode was always verified by ensuring that the latency of the electrically evoked N1 was approximately 0.6 ms. Recordings made from post-synaptic regions of the cochlear nucleus have considerably longer latencies due to the inherent synaptic delay. Stimuli evoking the N1E were generally restricted in current amplitude and pulse width due to the ease of corruption of the response via the electrical artefact.

Because of the significantly larger signal associated with recording N1E, compared with the EABR, system gain was reduced to a total gain of 80 dB. Only 10 responses were required for signal averaging. Otherwise the recording circuitry was identical to that used for EABRs.

Electrically evoked responses were recorded immediately following surgery, just prior and periodically during acute stimulation, and periodically following its completion. Threshold, latency and EABR/N1E input-output functions were determined by measuring the peak-to-peak amplitude of wave IV of the EABR or the electrically evoked N1 response (Chapter 2). Input-output functions recorded from the control cochlea, or recorded prior to acute stimulation in the stimulated cochlea, were obtained by averaging the peak-
to-peak amplitude of two sets of responses evoked by the
same stimulus. In these cases error bars indicate the
degree of variation between the responses. Per- and
post-stimulus response recordings were not duplicated due
to the time dependent nature of the experiment.

Per- and post-stimulus monitoring of the EABR
(or the N1E) were usually illustrated by expressing the
amplitude of the response as a percentage of its pre-
stimulus level. The post-stimulus recovery functions
were usually derived for three probe current levels†; a
probe current just above threshold, a probe current equal
to the stimulus current level used during acute
stimulation, and a third probe current at a level
significantly greater than the acute stimulus level.
This procedure allowed the response of the auditory nerve
to be monitored for three different evoked neural
populations as illustrated in Fig. 6.1.

† In the present study stimulus current refers to the
current level used during the period of acute
stimulation. Probe current refers to the current used
to evoke the EABR or N1E.
Figure 6.1 Schematic diagram of neural excitation distributions for three probe current levels evoked by a bipolar scala tympani electrode. The neural excitation distributions illustrated here represent three probe current levels: just above threshold, midrange, and a high probe current level. This procedure enabled the response of the auditory nerve to acute periods of electrical stimulation to be monitored for three different evoked neural populations.
6.3.5 Stimulus protocol.

Animals were stimulated at a fixed stimulus intensity and rate for a specific duration. On completion of the acute stimulation, the auditory nerve was allowed to recover. These recovery periods varied from 2-12 hours, depending on the nature of the experiment. During this recovery period EABRs (or N1p) were periodically monitored. Although recovery to pre-stimulus levels was observed in the majority of cases, there were a number of occasions where permanent stimulus induced reductions in the evoked response, were observed. In these cases subsequent periods of acute stimulation only proceeded following the recording of two consecutive stable responses, obtained at one hourly intervals.

In the present study, acute stimulus regimes varied from near maximum levels used clinically (e.g. 48 uC.cm⁻² geom. per phase; 200 pps), to stimulus levels significantly greater than these (e.g. 144 uC.cm⁻² geom. per phase; 1600 pps). Stimulus durations varied from 0.25 - 12 hours. Stimulus parameters systematically varied included charge density, current amplitude, total charge, stimulus rate, duration and duty cycle.

6.3.6 Strychnine.

In a number of experiments strychnine was used to block the cochlear efferents and to thereby examine
their contribution to the stimulus induced changes observed in the evoked response data. Strychnine was dissolved in sterile water for injection and administered i.p. at a dose of 0.5-1.0 mg/kg. Systemic administration of strychnine at this level has been shown to effectively block the cochlear efferent pathway without affecting cochlear afferent activity (Rajan and Johnstone, 1983). The administration of sterile water for injection served as a control in these experiments.

6.3.7 Anoxia.

The effects of anoxia on the stimulus induced changes in the EABR was studied in several animals. In these experiments the animal was intubated with a Portex® endotracheal tube. While the animal was breathing room air one cochlea was electrically stimulated at a fixed stimulus intensity and rate. During this time EABRs were periodically monitored bilaterally. The contralateral cochlea served as a control. Following collection of these baseline EABR data the animal was connected to a closed circuit ventilator (Medical Developments Pty. Ltd.) with a 100% nitrogen gas supply (CIG; High Purity Grade). After varying periods of anoxia the animal was allowed to recover by switching the ventilator to a 100% oxygen supply (CIG; Medical B.P. Grade). EABRs from both control and stimulated cochleas were recorded during and following the periods of anoxia.
6.3.8 Histological techniques.

Following completion of the acute stimulation program the animal was deeply anaesthetized with sodium pentobarbitone (200 mg; Euthatal®) i.p., and systemically perfused via the ascending aorta (having clamped the decending aorta) under gravitation. The right atrium was cut to allow the perfusate to flow freely. The initial perfusate was an isotonic, buffered, prewash solution. This was followed by a paraformaldehyde-glutaraldehyde fixative solution. Both perfusates were at 37°C (Appendix III details the histological procedure).

The temporal bones were removed and trimmed, and the round and oval windows opened. Holes were drilled into the apical turn of the cochlea and the semicircular canals to assist penetration of the fixative. Cochleas were then immersed in a glutaraldehyde based fixative at 4°C for 24 hours. During this time the otic capsule was trimmed. Cochleas were then decalcified in a glutaraldehyde based EDTA solution. Following decalcification the cochleas were thoroughly rinsed and then postfixed in a 1% OsO₄ solution. Finally, the specimens were dehydrated and embedded in Spurr epoxy resin (Agar Aids). During dehydration, the specimens were stained en bloc with uranyl acetate.

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Cochleas were sectioned at a thickness of 3 um using a Reichert-Jung Autocut® microtome. Sections every 240 um were collected and stained with haematoxylin and eosin. This tracer series was examined under the light microscope and the position of the electrode array within the scala tympani was determined using the graphical reconstruction techniques of Guild (see Chapter 4). Using this method, spiral ganglion cell populations adjacent to the bipolar electrode could be identified. Five micron thick sections of these regions were then cut and re-embedded onto a Spurr block. Under magnification, these specimens were trimmed to isolate specific regions of ganglion cells suitable for sectioning for TEM. Spiral ganglion cell populations both close and remote (i.e. middle and apical turns) from the electrode array were isolated for subsequent TEM analysis. Contralateral control cochleas were also prepared for ultrastructural examination.

These 5 um thick specimens were sectioned at a thickness of approximately 80-90 nm using an LKB III® ultramicrotome. These sections were placed on specimen grids and stained with lead citrate. The specimens were examined using an Hitachi HS-8 TEM operating at an accelerating voltage of 50 kV and a magnification range of 2,100-20,000 times.
6.4 Results.

6.4.1 Control cochleas.

In order to determine the stability of the electrically evoked responses, EABRs were recorded periodically from the contralateral control cochlea. A typical set of recordings is illustrated in Fig. 6.2. In this example, EABRs were evoked at five different probe current levels 60 hours following insertion of the electrode array. Wave IV, which was used to derive the input-output functions (Fig. 6.3), is arrowed. Wave I is obscured in this example due to the suppression of the stimulus artefact. Note the significantly longer wave IV latency associated with the 0.2 mA evoked response when compared with responses evoked at higher current levels. It is probable that at 0.2 mA the response is electrophonic in origin.

A typical set of input-output functions, recorded from the control cochlea of a normal hearing animal, is shown in Fig. 6.3. These responses were recorded periodically for the duration of the experiment (60 hours in this example). The long-term stability of the EABR, illustrated here, demonstrates the suitability of this experimental technique for recording EABRs over periods of 2-3 days. These stable responses were observed for both normal hearing and neomycin deafened control cochleas.
Figure 6.2 A series of EABRs recorded 60 hours following the surgical placement of the scala tympani electrode in control cochlea 365L. Wave IV is indicated by the arrow. The amplitude calibration line is 3 uV.
**Figure 6.3** EABR input-output functions recorded from control cochlea 365L. EABRs were recorded throughout the acute stimulation study. Recording times are in hours following insertion of the electrode array. The EABR series in Fig. 6.2 was used to construct the 60 hour input-output function in this figure.
6.4.2 Stimulus induced changes in the EABR.

(a) General observations.

Acute periods of electrical stimulation produced both per- and post-stimulus changes in the EABR. The extent of these stimulus induced changes depended on the stimulus intensity, rate and duration. In addition, these changes were also dependent on the intensity of the probe current used to evoke the EABR. In the present study the most extensive stimulus induced changes in the EABR were observed for responses evoked by probe currents less than the stimulus current.

An example of stimulus induced change in the EABR is illustrated in Fig. 6.4. In this example the cochlea was stimulated for a period of 1 hour at a stimulus intensity of 1 mA; 100 us per phase, at a rate of 200 pps. In this case the probe current used to evoke the EABR is the same intensity as the stimulus current. The initial response (0 min) was recorded just prior to the start of the 60 minute stimulus period. The next three responses (2, 6 and 60 min) were recorded during the acute stimulus period. A progressive decrease in the amplitude of the EABR and an increase in its latency, as a function of stimulation time, is apparent in these recordings. Moreover, these stimulus induced changes occur for all waves of the EABR. The final response (61.5 min) was recorded 1.5 minutes
following completion of the stimulus period. This response shows near complete recovery in EABR amplitude, however latency had not fully recovered to pre-stimulus levels.

More extensive per- and post-stimulus changes in the EABR are illustrated in Fig. 6.5. In this example stimulus intensity, rate and duration were greater than the previous example. Furthermore, the probe current used to evoke the EABR was less than the stimulus current level. In this example the cochlea was stimulated at an intensity of 1.8 mA; 200 us per phase, at a rate of 400 pps for a period of 4 hours. The initial response was recorded just prior to the 4 hour stimulus period. Significant reductions in the probe current elicited EABR occurred during the stimulus period. Moreover, post-stimulus recovery of both EABR amplitude and latency was significantly slower than observed in the previous example. In this case, the EABR amplitude took approximately 18 minutes to recover to its prestimulus level (4.3 hr; Fig. 6.5). As in the previous example, latencies had not fully recovered. Again, these stimulus induced changes were observed for all waves of the EABR.

Significant stimulus induced changes such as these, were observed for both normal hearing and neomycin deafened cochleas. Note the large difference in amplitude of the EABRs illustrated in these two examples.
Figure 6.4  FABRs recorded prior (0) during (2, 6 & 60 min) and following completion (61.5 min) of 1 hour of intracochlear stimulation at 1 mA; 100 us/phase and 200 pps. The probe current used to evoke the responses was the same intensity as the stimulus current.
Figure 6.5 EABRs recorded prior (0) during (1 & 4 hr) and following completion (4.1, 4.2 & 4.3 hr) of 4 hours of intracochlear electrical stimulation at 1.8 mA; 200us and 400 pps. In this example the probe current used to evoke the EABR was significantly less than the stimulus current. Note the change in the calibration bar between this and the previous example.
This is due, in part, to a significantly reduced neural population associated with 262N (Fig. 6.5) as a result of neomycin poisoning. Cat 365 was a normal hearing animal.

(b) **Stimulus intensity.**

The effect of varying stimulus intensity while maintaining stimulus rate and stimulus duration constant was examined in three animals. An example of the per-stimulus changes in EABR, observed for two different stimulus intensities, is illustrated in Fig. 6.6. In this example, change in EABR amplitude and latency (expressed as a percentage of their respective pre-stimulus response) is illustrated for two cochleas; one stimulated at a moderate stimulus intensity (347L; 0.8 mA, 200 us per phase), and the second at a high stimulus intensity (262L; 1.8 mA, 200 us per phase). In both cases stimulus rate and stimulus duration were constant. The probe currents used to evoke the per-stimulus EABRs were the same level as the stimulus.

In both cases EABRs showed evidence of a stimulus induced reduction in amplitude throughout the stimulus period. In these examples the per-stimulus response amplitude was approximately 80% of the pre-stimulus level. Although both cochleas in this example showed similar percentage reductions in EABR amplitude, differences were observed following completion of the
stimulus as illustrated in Fig. 6.7. Cochlea 347L, stimulated at a moderate stimulus level, exhibited rapid recovery in amplitude of the EABR. Indeed, post-stimulus amplitudes were greater than the pre-stimulus level. This "sensitization" period was often observed as part of the recovery process following acute stimulation at low and moderate stimulus intensities, and typically lasted for 20-30 minutes following completion of the stimulus, after which the EABR amplitudes returned to pre-stimulus levels. In contrast, cochlea 262L, which was stimulated at a high stimulus intensity, did not exhibit rapid post-stimulus recovery of the EABR amplitude (Fig. 6.7). Recovery to pre-stimulus levels was typically slow following stimulation at high stimulus intensities. In a number of cases EABR amplitudes failed to recover.

Stimulus induced increases in EABR latencies were observed for both cochleas in this example (Fig. 6.6), although the increase in latency observed for the moderately stimulated cochlea (347L) was only marginal. Significant per-stimulus increases in latency were observed for cochlea 262L. These latency observations, together with the amplitude data, indicate that more extensive stimulus induced changes in the EABR were associated with higher stimulus intensities. Unlike the amplitude data latencies immediately returned to pre-stimulus levels following completion of the stimulus.
Post-stimulus recovery functions for both cochleas are illustrated in Fig. 6.7. Only amplitude functions are illustrated here, as latencies showed rapid recovery to their respective pre-stimulus levels for both cochleas. The post-stimulus recovery functions shown in this figure have been derived for three probe current levels. This allows the post-stimulus recovery function for three evoked neural populations (i.e. less than, equal to and greater than the stimulus current level) to be determined.

Continuous stimulation at 0.8 mA and 200 us per phase, for a period of 12 hours at 100 pps, produced minimal post-stimulus changes in the amplitude of the EABR (347L, Fig. 6.7). At a probe current of 0.8 mA (the same level as the stimulus current) the EABR was initially 75% of its pre-stimulus level. Recovery was rapid. Within 90 seconds following completion of the 12 hour stimulus, EABR amplitudes evoked by this probe current were greater than pre-stimulus levels.

This rapid post-stimulus recovery of EABR latency differs from the two previous examples (Figs. 6.4 & 6.5). The stimulus regimes used in these examples included higher stimulus rates than the present example. Higher rates generally produced greater stimulus induced change in EABR latency (see 6.4.2).
Figure 6.6 Per-stimulus changes in EABR latency and amplitude for cochleas 347L and 262L. The amplitude and latency of wave IV of the EABR has been expressed as a percentage of its pre-stimulus level. Both cochleas were stimulated for 12 hours at a stimulus rate of 100 pps. 347L was stimulated at a moderate stimulus intensity (0.8 mA; 200 us/phase). In contrast 262L was stimulated using a high intensity stimulus (1.8 mA; 200 us/phase). The vertical bars in the figure indicate the duration of the stimulus period.
Figure 6.7 Post-stimulus recovery functions for cochleas 347L and 262L. In both examples the amplitude of wave IV of the EABR has been expressed as a percentage of its pre-stimulus level for three probe current evoked response levels.
347 Stimulated
64μC/cm² geom./phase
0.8 mA; 200 μs/phase
100 pps; 12 h

Recovery time (min)

Amplitude (%)

Probe
- 0.8 mA
- 1.4 mA
- 2.0 mA

252L Stimulated
144μC/cm² geom./phase
1.8 mA; 200 μs/phase
100 pps; 12 h

Recovery time (min)

Amplitude (%)

Probe
- 0.8 mA
- 1.2 mA
- 1.8 mA
This sensitization period lasted approximately 30 minutes after which the EABR returned to pre-stimulus levels, and was stable for monitoring periods of up to 720 minutes. Higher probe current levels (1.4 & 2.0 mA) showed little evidence of stimulus induced changes (although the 1.4 mA probe evoked response was routinely less than its pre-stimulus level).

In contrast, continuous stimulation at 1.8 mA and 200 us per phase at 100 pps over a 12 hour period produced significant long-term reductions in the amplitude of the EABR (262L, Fig. 6.7). At a probe current of 1.8 mA (the same current level as the stimulus), the EABR was again initially 75% of its pre-stimulus level. In this case, however, post-stimulus recovery was slow and failed to recover to pre-stimulus levels. Four minutes following completion of the stimulus, the EABR amplitude had recovered to approximately 80% of its pre-stimulus level where it remained relatively constant for the duration of the monitoring period. No evidence of sensitization was apparent. Lower probe current evoked responses (1.2 & 0.8 mA) showed more extensive stimulus induced changes. At these lower levels the evoked neural population would be within the population stimulated during the 12 hour stimulus period.
At a probe current of 1.2 mA, the amplitude of the evoked EABR was initially 25% of its pre-stimulus level. Although recovery to pre-stimulus levels was not complete, partial recovery showed evidence of a two phased recovery process. Initially there was evidence of rapid recovery, within 90 seconds following completion of the stimulus, to approximately 60% of the pre-stimulus level. During the next 10 minutes more gradual recovery was evident, with amplitudes recovering to 70% of the pre-stimulus level. Thereafter the EABR amplitude remained relatively stable.

At a probe current of 0.8 mA the initial EABR amplitude was 20% of the pre-stimulus level. In this example there was no evidence of a rapid recovery phase. Gradual recovery over a 30-60 minute period to approximately 45% was observed.

These permanent stimulus induced changes in the EABR indicate the adverse nature of this intense stimulus regime. Furthermore, recovery tended to occur within the first 60 minutes following completion of the stimulus. Thereafter, EABR amplitudes remained relatively constant for monitoring periods of up to 12 hours following the acute stimulus.
A second stimulus intensity series, recorded from one animal, is shown in Fig. 6.8. In this series, post-stimulus recovery functions were obtained following stimulation at 200 pps for 1 hour at stimulus intensities varying from 0.6-2.0 mA; 200 us/phase. Post-stimulus recovery of the EABR wave IV amplitude and latencies were recorded for three probe currents over a 120 minute period.

Following stimulation at 0.6 mA (Fig. 6.8a) only EABRs evoked by the lowest probe current (0.6 mA) showed evidence of stimulus induced changes. In this case both latency and amplitude recovered rapidly (within 90 seconds) to pre-stimulus levels. The amplitude data showed evidence of sensitization associated with this rapid recovery. At this stimulus level, EABRs evoked by higher probe currents (1.0 & 2.0 mA) showed no evidence of stimulus induced change.

Stimulation using progressively higher stimulus currents (1.0, 1.6 & 2.0 mA; Fig 6.8 b, c & d) showed an orderly increase in latency and decrease in amplitude of the post-stimulus evoked EABR. In addition, there was a progressive increase in the amount of stimulus induced change in EABRs evoked by higher probe currents (1.0, 1.6 & 2.0 mA). Furthermore, recovery periods to pre-stimulus levels, for both EABR latencies and amplitudes, increased with increasing stimulus levels.
Figure 6.8 EABR latency and amplitude recovery functions for 365R. This animal was stimulated at 200 pps for 1 hour at 4 different stimulus intensities (0.6, 1.0, 1.6 & 2.0 mA). In each case recovery of the EABR was recorded for 3 probe current levels over a 120 minute post-stimulus recovery period.
The effect of varying stimulus rate while maintaining stimulus intensity and duration constant was examined in four animals. A representative stimulus rate series is illustrated in Fig. 6.9. In this series post-stimulus recovery functions were obtained following stimulation at 1.0 mA and 200 us per phase for a period of 1 hour at stimulus rates varying from 100-1600 pps. Post-stimulus recovery of the EABR amplitude and latency (wave IV) were recorded for three probe currents (0.6, 1.0 & 2.0 mA) over a 120 minute recovery period. The order of stimulation rate presentation was selected at random. Furthermore, stimulation at a rate of 400 pps was repeated at the conclusion of the experiment in order to determine the degree of reproducibility.

Following stimulation at 100 pps, only EABRs evoked by the lowest probe current showed evidence of a stimulus induced change in sensitivity (Fig. 6.9a). Initially, the amplitude was approximately 75% of the pre-stimulus level, however the amplitude recovered rapidly to exhibit a period of considerable sensitization.

Like the intensity series data, stimulation using higher stimulus rates (200, 400, 800 & 1600 pps; Fig. 6.9 b-f) showed a progressive increase in latency and decrease in amplitude of the post-stimulus evoked EABR; longer post-stimulus recovery times to pre-stimulus
levels, and an increase in the amount of stimulus induced change in EABRs evoked by higher probe currents.

Stimulation at 200 pps (Fig. 6.9b) produced significant stimulus induced changes in the 0.6 mA probe evoked response. The initial post-stimulus amplitude was only 10% of its pre-stimulus level. Recovery was relatively slow (about 10 minutes) and there was evidence of a short sensitization period. The 1.0 mA probe evoked response showed significantly less stimulus induced change. The amplitude of the initial post-stimulus response was approximately 70% of its pre-stimulus level. For this probe level, recovery was rapid (< 90 s) after which the response was stable at pre-stimulus levels. The 2.0 mA probe evoked response showed no evidence of stimulus induced reduction in amplitude. There was, however, an indication of a short period of sensitization. Latency recovery functions showed less stimulus induced changes. Only the 1.0 mA probe evoked response showed an initial latency increase as this probe current response was recorded first.

Stimulation at 400 pps (Fig. 6.9 c & d) exhibited greater stimulus induced changes than were evident at 200 pps. Recovery of the 0.6 mA probe evoked response amplitude took 30 minutes. This was a significantly longer recovery period than experienced following stimulation at 200 pps. In addition, the 1.0 mA probe evoked response showed an initial significant
reduction in amplitude (8% of the pre-stimulus level), although recovery was rapid (within 90 s). There was no evidence of a stimulus induced reduction in amplitude of the 2.0 mA probe evoked response although there was evidence of slight sensitization over a 10 minute period. Latency data showed an initial post-stimulus increase for all probe current levels. At 0.6 and 1.0 mA probe levels, latencies took 3 minutes to recover to pre-stimulus responses. For the 2.0 mA probe current response, latency recovered rapidly (within 90 s).

Comparison of both sets of recovery function data following stimulation at 400 pps, shows very similar post-stimulus induced changes in the EABR.

Still greater stimulus induced changes in the EABR were observed at stimulus rates of 800 and 1600 pps (Fig. 6.9 e & f). In both cases the 0.6 mA probe evoked response exhibited only partial recovery of amplitude during the 120 minute post-stimulus monitoring period. Again, this recovery was slow and dependent on pulse rate. The maximum amplitude of recovery also depended on stimulus rate (70% at 800 pps and 60% at 1600 pps). The 1.0 mA probe evoked response also showed an increase in the post-stimulus amplitude recovery time (3 minutes at 800 pps and 6 minutes at 1600 pps). The 2.0 mA probe did not show a stimulus induced reduction although there was evidence of short-term sensitization (10-20 minutes) at these pulse rates.
Latencies for all probe current levels showed evidence of a stimulus induced increase following stimulation at 800 or 1600 pps. The rate of recovery was dependent on the probe current level. In both cases the 2.0 mA probe response exhibited rapid recovery (within 90 s). EABR recovery for the lower probe currents was somewhat longer. For example, the 0.6 and the 1.0 mA probe evoked responses took approximately 9 and 3 minutes respectively to recover following stimulation at 800 pps. Longer latency recovery periods were observed following stimulation at 1600 pps.

Clearly, stimulation using high stimulus rates (800 & 1600 pps) for this stimulus regime, produces significant long-term (and possibly permanent) changes in the excitability of probe evoked responses at low probe currents (which represent populations close to the stimulating electrodes). Stimulation at lower rates (200 & 400 pps) can produce significant temporary changes in excitability of these probe evoked neural populations.

It should be noted that although EABR amplitude data showed greater stimulus induced changes associated with responses evoked by low probe currents, greater initial post-stimulus changes in latency were frequently observed for the 1.0 mA probe evoked response. Since the 1.0 mA probe response was recorded prior to the 0.6 mA probe response, it is likely that there was considerable recovery in latency prior to the recording
Figure 6.9 EABR latency and amplitude recovery functions for 354R. This cochlea was stimulated at a fixed intensity of 1.0 mA and 200 us per phase for a period of 1 hour for 5 different stimulus rates (100, 200, 400, 800 & 1600 pps). Stimulation at 400 pps was repeated to determine the degree of reproducibility in the recovery functions. EABR recovery was monitored at three probe current levels (0.6, 1.0 & 2.0 mA) for a period of 120 minutes following completion of the stimulus.
Probe 1111 O.B IIA
1.0 lA 2.0 IIA
Recovery time (min)

Probe 1111 0.6
1.0 1M
100 ps: 1 h

Probe 1111 0.6
1.0 1M
2.0 1M
6-41

continued...
of the 0.6 mA probe response. At higher stimulus intensities - where more long-term recovery processes were evident - the rate of recovery was probe current dependent (e.g. Fig. 6.9 f). In these cases, longer latency recovery periods were associated with lower probe current evoked EABRs.

(d) **Stimulus duration.**

The effect of varying stimulus duration while maintaining stimulus intensity and stimulus rate constant was examined in three animals. Increase in stimulus duration (for a fixed stimulus intensity and rate) reduces the amplitude and increases the latency of the EABR (e.g. Fig. 6.4), although this stimulus parameter does not affect stimulus induced changes in the EABR to the same extent as stimulus intensity or stimulus rate.

An example of the reduction in amplitude of the EABR, as a function of stimulus duration, is illustrated in Fig. 6.10. In this example, post-stimulus recovery functions were obtained following stimulation at 1.5 mA; 200 pps for durations of 0.5 and 1 hour. Recovery functions were recorded for four probe current levels (0.5, 1.0, 1.5 & 2.0 mA) over a 120 minute post-stimulus recovery period. For all probe current levels, both latency and amplitude data showed slightly less stimulus induced changes for the shorter stimulus duration (0.5 hour; Fig 6.10a). The recovery of the 0.5 mA probe
evoked response is a good example. The initial post-stimulus amplitude response following 0.5 hours of stimulation, was approximately 10% of its pre-stimulus level. In contrast, following 1 hour of stimulation the same probe current level failed to evoke a response. Moreover, the 0.5 mA probe evoked response took 18 minutes to recover to pre-stimulus levels following 30 minutes of stimulation and 30 minutes following 1 hour of stimulation. The latency data also reflected more rapid post-stimulus recovery for the shorter stimulus period.

(e) Duty cycle.

The effect of reducing the duty cycle of the acute stimulation paradigm, on stimulus induced changes in the EABR, were studied in two experimental animals.

The stimulus protocol used is illustrated in Fig. 6.11. Scala tympani electrodes were stimulated at a rate of 400 pps (2.5 ms per current pulse) for a duration of 10 ms. A second 10 ms period followed during which there was no stimulation. This 50% duty cycle was repeated for the duration of the stimulus period (1 h). EABR post-stimulus recovery functions, recorded following this stimulus paradigm, were compared with similar post-stimulus recovery functions recorded following continuous stimulation for the same current levels and durations at both 200 and 400 pps (Fig. 6.12).
Figure 6.10  EABR latency and amplitude recovery functions for 411R. This cochlea was stimulated at a fixed intensity (1.5 mA; 200 us/phase) and rate (200 pps) for stimulus durations of 0.5 and 1 hour. EABR recovery was monitored at four probe current levels (0.5, 1.0, 1.5 & 2.0mA) for a period of 2 hours following completion of each stimulus.
All three stimulus paradigms exhibited significant temporary reductions in the amplitude of the post-stimulus EABR for low probe-current evoked responses. The amplitude of the initial post-stimulus response for the 0.6 mA probe current level was similar for all three stimulus paradigms (approximately 10% of their pre-stimulus response). Recovery was gradual and stimulus paradigm dependent. Complete recovery was observed 12 minutes following stimulation at 200 pps; 18 minutes following stimulation at 400 pps with a 50% duty cycle (400 pps(50%)); and 30 minutes following stimulation at 400 pps. The 400(50%) recovery function showed a close similarity with the 200 pps recovery function at higher probe currents (i.e. 1.0 & 2.0 mA).
Both initially showed an amplitude of 75% for the 1.0 mA probe. Moreover, recovery to pre-stimulus levels was rapid (within 90 s; Fig 6.12a&b). In contrast, the immediate post-stimulus response (at this probe level) following stimulation at 400 pps was approximately 5% of its pre-stimulus response (Fig. 6.12c). Furthermore, the recovery, although rapid, was somewhat longer than 90 seconds. Recovery functions for the 2.0 mA probe evoked responses were similar for the three stimulus paradigms, although there was some evidence of greater stimulus induced sensitization following continuous stimulation at 400 pps.

Post-stimulus latency recovery functions were consistent with the amplitude functions in showing greater similarity in the recovery process following stimulation at 200 pps and 400 pps(50%), compared with continuous stimulation at 400 pps (Fig. 6.12).

(f) Constant charge.

Previous research in this thesis has implied that total charge injection is not a significant stimulus parameter associated with stimulus induced neural damage mechanisms (Chapter Four). However, results from the present study indicate that increases in stimulus induced reductions in the EABR are a function of stimulus intensity and stimulus rate. Furthermore, the extent of these stimulus induced changes are reduced with a 50%
Figure 6.12  EABR latency and amplitude recovery functions for 354R, illustrating the effect of duty cycle on the post-stimulus recovery of the EABR. This cochlea was stimulated at a fixed intensity of 1.0 mA and 200 us/phase, for a period of 1 hour at a stimulus rate of 400 pps with a 50% duty cycle (a). This recovery function is compared with recovery functions following continuous stimulus regimes at 200 and 400 pps (b & c respectively). Note that stimulation at 400 pps(50%) would deliver the same number of current pulses per hour as continuous stimulation at 200 pps.
duty cycle stimulus regime. This suggests that increases in total charge injection may therefore correlate with increasing stimulus induced changes observed in the EABR.

To evaluate the effect of total charge injection on stimulus induced reductions in the EABR, two experimental animals were stimulated using the stimulus protocols illustrated in Table 6.1. For each constant charge series, four stimulus protocols were presented by varying stimulus intensity and rate or stimulus rate and duration while maintaining a constant total charge. Post-stimulus EABR recovery functions were recorded for periods of up to two hours following each protocol. The order of presentation of these protocols was selected randomly.

For both constant charge series the extent of stimulus induced changes observed in the EABR varied significantly among the stimulus protocols. Typical post-stimulus recovery functions for both constant charge series are illustrated in Figs. 6.13 and 6.14. Minimal post-stimulus reductions in EABR were recorded following stimulation using protocols where stimulus intensity and stimulus rate were both relatively low (e.g. protocol E; Fig 6.14a). Increasing stimulus intensity or stimulus rate effectively increased the extent of these stimulus induced changes although the nature of these changes was stimulus parameter dependent. The most extensive
Table 6.1  Stimulus protocols for constant charge series.

(i)  Stimulus duration constant.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Intensity†</th>
<th>Rate</th>
<th>Duration</th>
<th>Total Charge*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.0</td>
<td>200</td>
<td>1.0</td>
<td>288</td>
</tr>
<tr>
<td>B</td>
<td>1.5</td>
<td>266.7</td>
<td>1.0</td>
<td>288</td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
<td>400</td>
<td>1.0</td>
<td>288</td>
</tr>
<tr>
<td>D</td>
<td>0.5</td>
<td>800</td>
<td>1.0</td>
<td>288</td>
</tr>
</tbody>
</table>

(ii)  Stimulus intensity constant.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Intensity†</th>
<th>Rate</th>
<th>Duration</th>
<th>Total Charge*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1.0</td>
<td>100</td>
<td>2.0</td>
<td>144</td>
</tr>
<tr>
<td>F</td>
<td>1.0</td>
<td>200</td>
<td>1.0</td>
<td>144</td>
</tr>
<tr>
<td>G</td>
<td>1.0</td>
<td>400</td>
<td>0.5</td>
<td>144</td>
</tr>
<tr>
<td>H</td>
<td>1.0</td>
<td>800</td>
<td>0.25</td>
<td>144</td>
</tr>
</tbody>
</table>

†  Current phase constant at 200 us per phase.

*  Determined for one phase of the biphasic current pulse.
stimulus induced changes were observed following stimulation incorporating both relatively high stimulus intensities and rates (e.g. protocols A and B; Fig. 6.13 a&b).

Clearly, total charge injection cannot be implicated as a significant variable when assessing stimulus induced changes in the EABR. These reductions in excitability appear to depend on a complex relationship between stimulus intensity and stimulus rate.

As in previous results in the present study, stimulus induced reductions in EABR amplitudes (and corresponding increases in their latencies) were observed for responses evoked by probe currents equal to or less than the stimulus current level (e.g. Fig 6.13b). In addition, lower probe current evoked responses exhibited more extensive stimulus induced changes and longer post-stimulus recovery periods than responses evoked by higher probe current levels (e.g. Fig. 6.13a). EABRs evoked by probe currents greater than the stimulus level showed little relative change compared with their pre-stimulus levels (e.g. Fig. 6.13d).

The extent of these stimulus induced reductions was, however, a function of both stimulus intensity and stimulus rate. For example, following completion of 1 hour of stimulation at a stimulus level of 0.5 mA; 200 us per phase and a stimulus rate of 800 pps, the 0.5 mA
probe evoked response recovered to pre-stimulus levels within 1.5 minutes following completion of the stimulus (Fig. 6.13d). Higher probe current evoked responses showed little stimulus induced change in either amplitude or latency recovery functions. With increasing stimulus intensity (and a corresponding decrease in the stimulus rate) significantly greater stimulus induced changes were apparent (compare for example Fig. 6.13c&d). The most extensive stimulus induced changes observed in this series was recorded following 1 hour of stimulation at 1.5 mA; 200 us per phase and a rate of 267 pps (Fig. 6.13b). In this example the 1.5 and 1.0 mA probe evoked responses exhibited significant post-stimulus reductions in amplitude with a corresponding increase in recovery period to pre-stimulus levels (3 and 12 minutes respectively). The 0.5 mA probe evoked response only partially recovered (to 75% of its pre-stimulus level) during the 2 hour recovery period. These significant stimulus induced changes were also observed in the corresponding latency recovery functions.

Significantly, stimulation using protocol A (2.0 mA; 200 us per phase; 200 pps) - a higher stimulus intensity but lower rate than the previous example - resulted in less extensive stimulus induced changes (Fig. 6.13a). In this case all probe evoked responses had recovered to their per-stimulus levels within 9 minutes following completion of the stimulus regime.
Figure 6.13 EABR latency and amplitude recovery functions for cochlea 407R following stimulation using four protocols in which total charge was held constant while stimulus intensity and rate were systematically varied (Protocols A-D; Table 6.1). EABR recovery was monitored at four probe current levels for a period of 2 hours following completion of each protocol.
Recovery time (min)

Latency [%]

Amplitude [%]
Figure 6.14 EABR latency and amplitude recovery functions for cochlea 407R following stimulation using four protocols in which total charge injected was held constant while stimulus rate and duration were systematically varied (protocols E-H; Table 6.1). EABR recovery was monitored at four probe current levels for a period of 2 hours following completion of each protocol.
These findings, therefore, illustrate the importance of a combination of both high stimulus intensities and high stimulus rates (particularly rates greater than 200 pps) for significant stimulus induced change in the EABR. Furthermore, these results are consistent with previous results obtained in the present study showing that stimulus duration has a significantly less affect on stimulus induced changes in the EABR compared with stimulus intensity and rate.

Figure 6.14 also illustrates the increase in stimulus induced changes as a function of stimulus rate in a constant charge series where stimulus rate and duration were systematically varied.

(g) Effect of strychnine.

The effect of systemic administration of strychnine on stimulus induced reductions in the EABR was studied in two animals. In both cases strychnine, administered at a dose of 0.5-1.0 mg/kg (i.p.), had no observable effect on the stimulus induced changes in the EABR for monitoring periods of up to 30 minutes following its administration. The effects of administering similar doses of strychnine (i.p.) on acoustically generated N1 responses is generally quite apparent by this time (Rajan and Johnstone, 1983).
The lack of effect of strychnine on stimulus induced changes in the EABR is illustrated in representative results shown in Fig. 6.15. Per-stimulus EABR amplitude and latency functions were periodically recorded from both the stimulated and the contralateral control cochleae during 30 minutes of continuous stimulation at a fixed stimulus intensity and rate. Strychnine (i.p.) was administered following an initial 5.5 minutes of stimulation (indicated by the arrow in Fig. 6.15a). These per-stimulus changes in the EABR were compared with control data in which sterile water was administered in place of the strychnine solution, during stimulation using an identical stimulus regime (Fig. 6.15b). It should be noted that these control data were collected prior to the strychnine administration.

In both per-stimulus functions (Fig. 6.15a&b) the stimulated cochlea exhibited a rapid increase in EABR latency (within the first minute of stimulus onset), and a somewhat more gradual decrease in EABR amplitude. With increasing stimulus duration EABR latency, in both examples, continued to increase gradually to a maximum of approximately 130% of their pre-stimulus levels. Simultaneously, EABR amplitudes decreased - although these changes were more gradual than the latency changes - to minimum levels of 40-45%. These per-stimulus changes were similar in both cases despite the
Figure 6.15 (a) Per-stimulus EABR latency and amplitude functions recorded from both the stimulated (▲) and the control (△) cochleae of cat 421. These data were recorded during 30 minutes of stimulation at 1.5 mA; 200 us per phase and a rate of 200 pps. Strychnine (0.5 mg/kg, i.p.) was administered 5.5 minutes following the start of the stimulus period (arrowed).

(b) Control per-stimulus amplitude and latency functions recorded from the same animal using an identical stimulus regime. Sterile water (5ml, i.p.) was administered 5.5 minutes following the start of the stimulus period (arrowed).
administration of strychnine. Unstimulated control cochleas showed little change in either EABR latency or amplitude during the 30 minute monitoring period.

(h) **Effect of anoxia.**

The effects of anoxia on stimulus induced changes in the FABR was studied in four animals. Representative results from one experiment are illustrated in Fig. 6.16. In these examples per-stimulus EABR latency and amplitude functions were periodically recorded from both the stimulated and the contralateral control cochleae during a period of continuous stimulation at a fixed stimulus intensity and rate (Fig. 6.16a). Following an initial 10.5 minute stimulation period the animal was made anoxic by connecting it to a 100% nitrogen gas supply via a closed circuit ventilator. The animal was exposed to nitrogen - in this example for a period of 10 minutes - after which it was allowed to recover by switching the ventilator to a 100% oxygen supply. The nitrogen respiration period is indicated by parallel arrows in Fig. 6.16a.

Typical per-stimulus FABR latency and amplitude functions were observed prior to nitrogen respiration. FABRs increased to approximately 120 - 125% of pre-stimulus levels while amplitudes decreased to 45 - 55%. Both latency and amplitude functions had stabilized prior to the introduction of nitrogen.
Per-stimulus data from the control cochlea was stable throughout this period.

Per-stimulus latency and amplitude functions for both stimulated and control cochleae were influenced by anoxia. The extent of these changes increased with the duration of the nitrogen respiration. Significantly, these anoxia induced changes were always observed in the stimulated cochlea two to three minutes before they became apparent in the control cochlea. Moreover, in cases where there was recovery following anoxia, the magnitude of these anoxia induced changes were greater for the stimulated cochlea when compared with its contralateral control (e.g. Fig 6.16a).

These anoxia induced changes in per-stimulus EABRs (i.e. reductions in amplitude and a corresponding increase in latency), were often preceded by a limited sensitization period in which EABR amplitudes increased and latencies decreased. These short periods of sensitization were observed for both control and stimulated cochleae (e.g. Fig. 6.16a). Immediately following these limited periods of sensitization rapid anoxia induced reductions in EABR amplitude and increases in EABR latency occurred for both the stimulated and, subsequently, the control cochleae.
Figure 6.16 Effects of anoxia on EABR per-stimulus and post-stimulus recovery functions. (a) Per-stimulus latency and amplitude functions were recorded for both the stimulated (▲) and control (△) cochleae. Following a 10.5 minute stimulus period to allow the per-stimulus functions to stabilize, the animal was made anoxic for a period of 10 minutes (arrowed). In this example anoxia influenced the behaviour of both the control and stimulated per-stimulus EABRs. (b) Post-stimulus recovery functions following 30 minutes of stimulation during which time the animal was made anoxic for a period of 10 minutes. Anoxia significantly reduced the rate of recovery of the post-stimulus EABR.
A stimulated control

Stimulus time (min)

Recovery time (min)

Wave IV Latency [ms]

Wave IV Amplitude [%]

Latency [ms]

Amplitude [%]

Recovery time (min)

421 anoles
78 µA/cm² gases/phase
1.5 mA 200 µs/phase
200 ppm 0.5 h

421 anoles 0.5 h
78 µA/cm² gases/phase
1.5 mA 200 µs/phase
200 ppm 0.5 h

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In animals in which post-anoxia recovery was examined (e.g. Fig 6.16a), per-stimulus amplitude and latency functions tended to recover to pre-anoxia levels for unstimulated control cochleas. In stimulated cochleas, amplitude functions in particular, failed to recover to pre-anoxia levels during stimulation. The extent of this partial recovery was inversely related to the duration of anoxia. Unusually long post-stimulus amplitude and latency recovery functions were observed following periods of acute anoxia. An example is illustrated in Fig 6.16b. The extended and incomplete recovery functions illustrated in this example is in contrast to recovery following stimulation using an identical stimulus regime in the absence of anoxia, in which complete recovery - for all probe current evoked responses - was observed within 10 minutes following completion of the stimulus (Fig. 6.10a).

6.4.3 Stimulus induced changes in N1E.

(a) General observations.

Stimulus induced changes in the electrically evoked N1 were recorded from three animals in the present study. A typical set of recordings is illustrated in Fig. 6.17. In this example the N1E was evoked at five different probe current levels using a 100 us per phase biphasic current pulse. As observed with the EABR,
increasing the intensity of the probe current will increase the amplitude and decrease the latency of the electrically evoked N1 response. The changes in N1E with increasing probe current closely reflect the changes observed in wave IV of the EABR (e.g. Fig. 2.23).

Acute periods of electrical stimulation produced both per- and post-stimulus induced changes in the N1E. As observed with the EABR, the extent of these stimulus induced changes depended on stimulus intensity, rate, duration and the intensity of the probe current used to evoke the N1E.

An example of stimulus induced change in the N1E is illustrated in Fig. 6.18. In this example the cochlea was stimulated for a period of 1 hour at a stimulus intensity of 1.8 mA; 150 μs per phase, at a rate of 200 pps. The initial response (0 min) was recorded just prior to the start of the 60 minute stimulus period. The next three responses (2, 6 & 50 min) were recorded during the acute stimulus period. A decrease in the amplitude of the N1E and an increase in its latency is apparent in these recordings. The final response (61 min) was recorded 1 minute following completion of the acute stimulus. This response shows complete recovery to pre-stimulus levels. The behaviour of the N1E both during and following acute intracochlear electrical stimulation therefore appears similar to that observed with the EABR (e.g. Fig. 6.4).
Figure 6.17 A series of electrically evoked N1 responses recorded from a pt ball electrode placed on the auditory nerve.
**Figure 6.18** A series of N1Ps recorded prior (0), during (2, 6 & 50 min), and following completion of 1 hour of intracochlear stimulation at 1.8 mA; 150 us per phase and 200 pps. The probe used to evoke the response is the same level as the stimulus.
The relationship between stimulus induced changes in the N1F and the EABR.

The relationship between stimulus induced changes in the N1F and wave IV of the EABR was studied in one animal by recording both evoked responses during and following completion of a common stimulus regime. The per-stimulus changes are illustrated in Fig. 6.19. In this example, changes in both amplitude and latency of the N1F and the EABR wave IV, have been determined during 1 hour of stimulation at a stimulus intensity of 2.0 mA; 200 us per phase, and at a stimulus rate of 200 pps. Both evoked potentials showed evidence of a stimulus induced reduction in amplitude throughout the stimulus period. Moreover, the extent of this reduction was similar for both the N1F and the EABR; in this example the per-stimulus response amplitude was approximately 70% of the pre-stimulus level for both the N1F and the EABR after 60 minutes of stimulation (Fig. 6.19). Similarly, stimulus induced increases in latencies were observed for both the electrically evoked N1 and the EABR, although the N1F exhibited a significantly greater percentage increase in latency (the absolute increase in latency was less than observed for wave IV of the EABR. The relative increase was greater due to the short latency of the N1F).

An example of post-stimulus recovery functions for both N1F and wave IV of the EABR are illustrated in
Fig. 6.20. Only amplitude functions are illustrated here. Furthermore, 2.0 mA probe evoked recovery functions are not shown because the rapid post-stimulus reduction in the latency of $N1_E$ resulted in contamination by the electrical artefact. From the figure it is apparent that the post-stimulus recovery of $N1_E$ is reflected in the recovery of wave IV of the EABR. Moreover, this recovery process is probe current dependent, with lower probe current evoked responses (0.6 mA; Fig. 6.20) taking a longer period of time to recover than probe evoked responses at higher probe current levels (1.0 mA; Fig. 6.20).

The per- and post-stimulus changes in $N1_E$ are, therefore, similar to the changes observed for wave IV of the EABR, and imply that stimulus induced changes observed in the EABR are dominated by changes in excitability at the level of the auditory nerve. These results are consistent with previous observations describing the relationship between EABR and $N1_E$ input-output functions (section 2.11.2 (c)), indicating that wave VI of the EABR accurately reflects the behaviour of $N1_E$ with little apparent modification of the $N1_g$ amplitude and latency responses occurring within the auditory brainstem generators. Similar findings have been reported for acoustically evoked N1 and wave VI of the ABR (Huang and Buchwald, 1978).
Figure 6.19 Per-stimulus latency and amplitude functions for both the N1e and the EABR of cochlea 365R. The evoked responses were recorded during intracochlear electrical stimulation for a period of 1 hr at an intensity of 2.0 mA; 200 μs/phase and a stimulus rate of 200 pps.
Figure 6.20 Post-stimulus amplitude recovery functions for both the N1P and the EABR of cochlea 365R following 1 hr of continuous stimulation using a common stimulus regime. The recovery functions illustrated represent two probe current levels (0.6 and 1.0 mA).
Ultrastructural results.

Control and stimulated cochleas from six representative animals in the present study were examined for evidence of ultrastructural change to the spiral ganglion cells. Unless otherwise stated, spiral ganglion cells illustrated here are from the basal turn of the cochlea adjacent to the scala tympani electrode array. Evidence of ultrastructural change that could be attributed to electrical stimulation was only observed in one cochlea.

Spiral ganglion perikaryon from the basal turn of implanted non-stimulated control cochleas exhibited normal ultrastructural morphology. A representative micrograph is illustrated in Fig. 6.21, and shows a Type I spiral ganglion perikaryon from the basal turn of control cochlea 213R. The cytoplasm is normal in appearance, containing a large number of ribosomes and granular endoplasmic reticulum, mitochondria and an occasional Golgi body. The large, round, centrally located nucleus contains a fine evenly dispersed chromatin and a prominent electron dense nucleolus. A thin myelin sheath envelopes the cell body and is associated with a normal Schwann cell. Similar ultrastructural morphology was observed in all but one of the stimulated cochleas examined. A representative
example is shown in Fig. 6.22. In this case the stimulated cochlea (374R) exhibited no stimulus induced change in the EABR.

Evidence of ultrastructural disruption of organelles within the spiral ganglion were observed in one electrically stimulated cochlea (262L). Significantly, this cochlea was stimulated at a high intensity and rate producing a permanent stimulus induced reduction in the EABR (Fig. 6.7). This reduction was the most extensive observed in the present study. It should be noted that this animal had been deafened with neomycin which could also have contributed to these ultrastructural changes. Morphological changes in the form of ballooning and disruption of mitochondria, and a reduction in the ribosome content of the cytoplasm, were observed in the perikaryon of spiral ganglion cells adjacent to the stimulating electrode (Fig. 6.23 & 6.24). These ultrastructural changes were not a result of preparation artefact as adjacent Schwann cells exhibited normal mitochondria (Fig. 6.23). Moreover, ganglion cells more apical to this region as well as ganglion cells from a similar region in the contralateral control cochlea, appeared normal (Figs. 6.25 & 6.26).
Figure 6.21 Electron micrograph showing a Type I spiral ganglion perikaryon from the basal turn of control cochlea 213R. This cochlea was acutely implanted with a scala tympani electrode but was not electrically stimulated. The cytoplasm is rich in ribosomes, granular endoplasmic reticulum (ER), and mitochondria. Occasional Golgi body (G) are also present. The lighter nucleus contains a central, electron dense nucleolus. A thin myelin sheath (M) envelopes the perikaryon. A Schwann cell is also present (S). x6,100

Figure 6.22 Electron micrograph showing a Type I spiral ganglion perikaryon from the basal turn of the stimulated cochlea 374R. This cochlea was acutely stimulated but exhibited no permanent stimulus induced reductions in the EABR. The cytoplasm and nucleus are similar in organization and content to the control illustrated above. Note the longitudinal orientation of the neurofilaments near the axon hillock. x4,500
**Figure 6.23** Electron micrograph of a portion of a Type I spiral ganglion perikaryon from the basal turn of stimulated cochlea 262L. This cochlea was acutely stimulated at high intensities and rates, producing permanent stimulus induced reductions in the EABR. The majority of mitochondria within the perikaryon exhibited ballooning or extensive membrane disruption (arrow). There is also a significant reduction in the ribosome content within the cytoplasm. Mitochondria in an adjacent Schwann cell ($M_8$) appear normal. \[x16,000\]

**Figure 6.24** Electron micrograph of a Type II spiral ganglion perikaryon taken from the same region as the example in Fig. 6.23. This cell body was not enveloped by a myelin sheath. As in the previous example, the majority of mitochondria are disrupted (arrow). The ribosome content is low although the cytoplasm appears to contain more filamentous structures typical of Type II ganglion cells. A Golgi body appears normal. \[x16,000\]
**Figure 6.25** Electron micrograph of a Type I spiral ganglion perikaryon taken from a more apical region of the basal turn than the examples shown in Figs. 6.23 & 6.24 and is a greater distance from the stimulating electrode array. The ultrastructural arrangement of this cell appeared normal. D, dark inclusion body; E, granular endoplasmic reticulum; G, Golgi body; M, myelin sheath; S Schwann cell. x8,500

**Figure 6.26** Electron micrograph of a Type I spiral ganglion perikaryon taken from the basal turn of the contralateral control cochlea 262R. The ultrastructural arrangement of this cell appeared normal. D, dark inclusion body; E, granular endoplasmic reticulum; G, Golgi body; M, myelin sheath; N, nucleus. x13,000
6.5 Discussion

Results of the present study have shown that electrical stimulation of the auditory nerve using pulsatile electrical stimuli can produce reversible and, under certain stimulus conditions, irreversible reductions in the excitability of auditory nerve fibres. Moreover, in cases where irreversible changes in neural excitability were recorded, there was some evidence of ultrastructural disruption within the soma of the primary auditory neurones. The extent of these stimulus induced changes were stimulus intensity and stimulus rate dependent. Although other stimulus parameters such as total charge, did not appear to contribute to these changes, the present results nevertheless indicate that stimulus parameters other than charge density must be considered when assessing stimulus induced neural damage mechanisms. Moreover, the nature of these stimulus induced changes implicates damage mechanisms associated with excessive neural activity and the depletion of available metabolites. Such damage mechanisms have not previously been considered when assessing the safety and efficacy of neural stimulation.

The extent of stimulus induced reductions in the excitability of the auditory nerve, illustrated in the present study, were determined by recording reductions in the amplitude and increases in latency of both the $N_{I_E}$ and wave IV of the ERAB. Comparison of stimulus induced
changes in both the N1p and the EABR to a common stimulus, showed that wave IV of the EABR simply reflected the changes occurring in the N1p (Figs. 6.19 & 6.20). Such observations imply that these stimulus induced changes in excitability are restricted to the level of the auditory nerve. Subsequent EABR generators do not appear to contribute to these changes. This is supported by the observation that stimulus induced changes in the EABR occur for all waves with subsequent waves closely following the changes observed in wave I (Fig. 6.4). Other authors investigating effects of electrical stimulation on both peripheral and central neural pathways have also concluded that stimulus induced changes in neural excitability originate close to the site of electrical stimulation (Ignelzi and Nyquist, 1976; McCreery et al., 1986), and are not associated with reductions in post-synaptic excitability.

The extent of these changes were dependent on stimulus intensity, stimulus rate and the level of the probe current. Evoked populations from low probe current levels exhibited greater stimulus induced changes compared with populations evoked from higher probe currents. This consistent observation implies that greater reductions in excitability were associated with neural populations closer to the stimulating electrodes. Such a population would be subjected to a
greater degree of evoked neural activity compared with populations more remote from the stimulating electrodes. The concept that neural activity is associated with the extent of these stimulus induced changes is supported by the effect of duty cycle on stimulus induced change (Fig. 6.12). These observations form the basis of the neural activity model used to explain the mechanisms underlying both the temporary and permanent stimulus induced changes in neural excitability.

Physiological and modelling studies have shown that current and neural excitation distributions produced by bipolar scala tympani electrodes are bell shaped with gradually decaying boarders (Black and Clark, 1980; Black et al., 1983; Lukies et al., in press). These bell shaped distributions are apparently symmetrical about the centre line midway between the two electrodes constituting the bipolar pair. Schematic representation of these neural excitation distributions is shown in Fig. 6.27a. At low stimulus levels the electrical stimulus excites a relatively small, localized neural population midway between the bipolar electrode pair. For the majority of these fibres the stimulus level is just above threshold. Therefore these units will exhibit a relatively broad degree of synchronization and will not fire in response to every stimulus pulse.
Increasing the stimulus intensity increases both the current and neural excitation distributions (Fig. 6.27a). The neural population previously excited by near threshold stimuli now exhibits increasingly greater degrees of deterministic firing. Single auditory neurone recordings in response to electrical stimulation have consistently demonstrated that the degree of this highly synchronized neural activity is both stimulus intensity and rate dependent (Moxon, 1971; Merzenich et al., 1973; Hartmann et al., 1984; van den Honert and Stypulkowski, 1984; Javel et al., in press). This highly deterministic activity is rate dependent because greater stimulus intensities are required to sustain highly synchronized neural activity as pulse rates increase (Moxon, 1971; Javel et al., in press). At sufficiently high stimulus intensities electrically evoked spikes are capable of firing in response to every stimulus pulse even for pulse rates of up to 600 to 800 pps (Javel et al., in press).

For a given stimulus level and rate, however, the ability of neurones to fire in a highly deterministic manner will reduce with increasing distance from the midline of the neural excitation distribution. This variability in deterministic firing, for a fixed stimulus intensity, is illustrated schematically in Fig. 6.27b. Three period histograms depict varying degrees of neural synchronization to the stimulus pulse within this
electrically evoked neural population. Fibres on the periphery of the neural excitation distribution exhibit relatively poorly synchronized activity, firing only intermittently in response to the stimulus. Proceeding more centrally within this distribution function fibres exhibit an increasing degree of synchronized activity. Spike rates increase as fibre activity becomes more deterministic. The presence of two peaks in this period histogram implies that this neurone is being excited, alternatively, at two distinct sites. Presumably the short latency peak represents excitation at the axon while the latter peak represents excitation at the peripheral dendrite (Javel et al., in press). Finally, the most central fibres within this distribution function exhibit highly deterministic activity in response to the stimulating pulse. The extent of neural activity for any neurone within this excitation distribution is therefore dependent on stimulus intensity, stimulus rate and the neurones spatial location with respect to the stimulating electrodes. Fibres positioned centrally within this bell shaped distribution would exhibit greater electrically evoked activity in comparison with fibres located at the distribution periphery. Naturally, neurones outside this distribution function would not exhibit driven activity in response to the stimulus.
Figure 6.27  (a) Schematic diagram of neural excitation distributions for three current levels evoked by a bipolar scala tympani electrode.

(b) Variability of neural activity among a population of auditory nerve fibres electrically stimulated via a bipolar scala tympani electrode. Period histograms (adapted from Javel et al., in press) illustrate the degree of synchronized neural activity for three neurones within the neural excitation distribution. Number of spikes is plotted along the ordinate and time (in ms) along the abscissa of the period histograms which were recorded in response to current pulses presented at 200 pps.
auditory nerve

scala tympani electrodes

b
This neural activity model was tested by spatially altering the stimulating and probe electrode geometries as illustrated in Fig. 6.28. An electrode array containing four Pt band electrodes was inserted into the scala tympani of a normal hearing cat. All post-stimulus recovery functions were recorded following stimulation using a common stimulus regime (1.5 mA, 200 µs/phase, 1 h). Three representative post-stimulus recovery functions are illustrated in Fig. 6.28.

Post-stimulus recovery evoked by probe electrode pair 1-4 following stimulation using the same bipolar pair is shown in Fig. 6.28b. Note that this electrode pair is the widest combination on the electrode array. Post-stimulus recovery functions obtained with this configuration were, in general, similar to recovery functions recorded from other animals using similar stimulus regimes (e.g. Fig. 6.8c). Greater reductions were, however, noted in the amplitude recovery functions evoked by the 1.5 and 2.0 mA probe currents. In fact this was the only example where a probe current, which was greater than the stimulus current, evoked a neural population that exhibited evidence of a stimulus induced reduction in excitability (2.0 mA probe, Fig. 6.28b). It is probable that this was associated with the wide spacing of this bipolar electrode configuration. Nevertheless, the basic recovery functions illustrated in
this example were similar to previous recovery functions where stimulating and probe electrodes were confined to the one electrode pair.

In the second example (Fig. 6.28c) the probe electrode pair was altered to the spatially restricted pair 2-3. As in the previous example the cochlea was stimulated using electrode pair 1-4. Significantly, recovery functions for all probe current levels using this probe electrode configuration exhibited extensive stimulus induced changes in EABR amplitude. These stimulus induced changes were the most extensive reversible changes observed in the present study. Significant reductions in the 2.0 mA probe evoked response, for example, took 9 minutes following completion of the stimulus to recover to pre-stimulus levels although this probe current was somewhat greater than the stimulus current level (1.5 mA). Increases in latency were also observed however they tended to recover more rapidly than the amplitude functions.

In contrast, the third example illustrates the extent of post-stimulus changes in EABR following stimulation with bipolar configuration 2-3 while monitoring post-stimulus recovery with pair 1-4 (Fig. 6.28d). In this example post-stimulus changes were restricted to the 0.5 mA probe current evoked response. Although the stimulus current level was 1.5 mA, probe evoked responses at probe currents of 1.0, 1.5

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and 2.0 mA showed no evidence of stimulus induced change. Such recovery functions are not observed when the stimulating and probe electrodes are one and the same.

These results clearly demonstrate the presence of a spatially dependent neural population exhibiting varying degrees of stimulus induced reductions in neural excitability. Further, the most significant changes appear to be associated with neurones positioned midway between the bipolar stimulating electrodes. Less extensive stimulus induced changes appear in neural populations more distally located from this midline. These experimental observations support the neural activity model discussed previously.

Post-stimulus recovery functions recorded in the present study routinely exhibited a two-phased recovery process. Initially a rapid and frequently extensive recovery was observed within the first 90 seconds following completion of the stimulus regime. This rapid recovery phase was most apparent for recovery functions where the probe and stimulus current levels were the same (e.g. 0.5 mA probe, Fig 6.13d). In these cases the initial post-stimulus recovery function was recorded immediately following completion of the stimulus regime. More limited evidence of this rapid recovery phase was apparent for recovery functions elicited by probe currents which were less than the stimulus current.

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Figure 6.28 Effects of spatial variations in the neural excitability distribution on the extent of stimulus induced changes in the auditory nerve. (a) Schematic diagram of the four ring scala tympani array used in this study illustrating spatial variations in the neural excitability distribution for two bipolar electrode configurations. Post-stimulus recovery functions were recorded following stimulation using a common stimulus regime. A variety of stimulus and probe electrode combinations were used: (b) stimulating and probe electrode 1-4; (c) stimulating electrode 1-4, probe electrode 2-3; (d) stimulating electrode 2-3, probe electrode 1-4.
auditory nerve

scala tympani electrodes

Probe pair I-4
- 0.5 mA
- 1.0 mA
- 1.5 mA
- 2.0 mA

Recovery time (min)

Latency (ms)
In these cases the initial probe current evoked response was recorded up to 30 - 40 s following completion of the stimulus regime and presumably after significant recovery of this rapid recovery phase. Lower probe currents (relative to the stimulus current level) exhibited progressively less evidence of this rapid recovery phase and greater evidence of more long-term recovery. The extent of the rapid recovery phase was also dependent on the stimulus regime. Increasing stimulus intensity and/or rate increased the extent of the long-term recovery phase at the expense of this rapid phase.

While this post-stimulus monitoring procedure was restricted to recording gross changes in the rapid recovery of the EABR over a period of 60 - 90 seconds, observations made during the experimental series indicated that the majority of recovery associated with this short-term phase occurred very rapidly (within the first few seconds) following completion of the stimulus.

The second, long-term recovery phase was active for periods of up to 1-2 hours (e.g. Fig. 6.9e). This long-term recovery was also dependent on probe current level and stimulus regime. Longer recovery periods were associated with lower probe current responses and more intense and/or more rapid stimulus regimes. The present results indicated that maximum recovery occurred within a 1-2 hour post-stimulus period after which minimal recovery was observed - even for monitoring periods of up to 12
hours following completion of the stimulus regime. Incomplete recovery was occasionally observed (e.g. Fig. 6.7). Such long-term changes in neural excitability were considered permanent.

The presence of two distinct recovery phases within the post-stimulus recovery function implies that there are at least two mechanisms underlying these stimulus induced changes. These recovery processes probably reflect short and long-term metabolic changes in the stimulated neural population.

There are at least two possible mechanisms that can explain the rapid recovery phase observed immediately following completion of the acute stimulation regime. Both mechanisms result in a reduction in neural excitability associated with local transient changes in ionic concentrations within and immediately surrounding the neurone as a result of the propagation of an action potential. The extent of these changes in excitability depends, in part, on the extent of previous neural activity. Furthermore, both mechanisms exhibit rapid recovery following completion of the tetanizing stimulus.

Repetitive stimulation, at a rate where the period of stimulation is longer than the fibre's relative refractory period, will not produce a change in the size of the fibre's response. If, however, the stimulus rate is increased so that the stimulus period falls within the relative refractory period, the amplitude of the response
of subsequent spikes becomes smaller and their conduction velocity is reduced (Forbes and Rice, 1929; Gasser, 1935). Stimuli falling earlier within a fibre's relative refractory period evoke progressively smaller spikes until a stimulus rate is reached at which the stimulus becomes subthreshold.

Increasing the relative refractory period is dependent on fibre activity. Stimulating a neurone with a train of current pulses will increase that neurones relative refractory period. The extent of this increase will depend on the intensity, rate and duration of the stimulus. The effect of acute periods of electrical stimulation will, therefore, result in increases in the relative refractory period of activated fibres within the auditory nerve. Because increases in the relative refractory period are activity dependent, the greatest increases will be associated with low threshold fibres and stimulus regimes of high stimulus intensity, rate and duration.

These increases in the relative refractory period would place some neurones subthreshold, and decrease the neural conduction velocity of fibres still being activated. Such changes in neural excitability and conduction velocity adequately accounts for the per-stimulus EABR amplitude and latency changes observed in the present study (e.g. Fig. 6.6).
It is another matter, however, for increases in the refractory state to contribute to the rapid phase of the post-stimulus recovery function. For the relative refractory period to be a contributing factor to this short-term recovery process, the probe stimulus evoking the EABR must fall within the fibre's refractory period. Since EABRs were routinely recorded at stimulus periods of 30 ms it would be necessary for the refractory period to be somewhat greater than this if it were to have any significant effect on neural excitability. This period is much greater than the relative refractory period of a resting mammalian auditory nerve fibre (approximately 1-2 ms; Moxon, 1967). While tetanizing stimuli can substantially increase the duration of a fibre's refractoriness (Forbes and Rice, 1929), it is most unlikely to extend to 30 ms or more. It is therefore unlikely that refractoriness contributed to the rapid recovery recorded immediately following completion of the stimulus regime.

A more probable mechanism is associated with the reduction in neural excitability following increases in the amplitude of the positive afterpotential. Both the positive and negative afterpotentials are small amplitude, long duration changes in neural membrane polarization. Both afterpotentials are observed after the propagation of an action potential, although their form varies considerably from one fibre type to
another. Significantly, they are particularly dependent on the previous activity of the fibre and changes in the fibre's metabolic condition (Brinley, 1980).

The negative afterpotential occurs immediately following the action potential and is basically a residual depolarization of the neural membrane that persists for a few milliseconds. The smaller amplitude positive afterpotential is observed following the negative afterpotential. During this period the neural membrane becomes slightly hyperpolarized and may last, in mammalian nerves, for periods ranging from 30 - 200 ms. The duration of the positive afterpotential may, however, be significantly lengthened during periods of neural activity. Moreover, although the amplitude of the positive afterpotential is very small after the passage of a single action potential, a tetanizing stimulus produces an accumulated increase in this amplitude in proportion to the number of spikes propagated. This effect is observed experimentally as post-tetanic hyperpolarization (Gasser, 1935; Brinley, 1980). Significantly, the duration of the positive afterpotential generally correlates with a period of subnormal neural excitability and a decrease in the conduction velocity (Gasser, 1935; Brinley, 1980). Unlike the positive afterpotential, the negative afterpotential does not appear to increase in amplitude during periods of neural activity.
It is possible then, that the positive afterpotential of stimulated auditory nerve fibres increased in both amplitude and duration throughout the acute stimulation regime. EABRs, recorded immediately following completion of the stimulus, would show a reduction in neural excitability as the probe stimulus would fall within the positive afterpotential evoked by the previous stimulus. The greater the degree of neural activity the greater the reduction in the initial post-stimulus response due to the accumulative nature of the positive afterpotential. Probe current levels less than the acute stimulus would evoke significantly smaller responses due to the increase in response threshold. Conversely, probe currents greater than the stimulus level would evoke near normal response amplitudes as the nerve would only be subnormal in threshold during the positive afterpotential. Nerve fibres would respond normally to stimulus currents greater than threshold. These stimulus induced changes - resulting in changes in the positive afterpotential - are consistent with the changes in excitability observed experimentally.

This initial rapid recovery phase appears to be complete within the 90 second monitoring period suggesting that the positive afterpotential recovers rapidly following a tetanizing stimulus. Gasser (1935) has reported a two component recovery process for the positive afterpotential of the frog sciatic nerve. He
termed these components P1 and P2. P1 was of short
duration, diminishing in the order of seconds following
completion of a tetanizing stimulus. P2 was of longer
duration, having an effect on neural excitability 10 - 15
minutes following completion of the stimulus. The short
duration P1 component observed by Gasser is consistent
with the rapid recovery of the early post-stimulus phase
observed in the present study. It may be that the more
long-term recovery process evident in the present study
included a component equivalent to the P2 component
described by Gasser.

Although stimulus induced changes in the
positive afterpotential can explain the rapid recovery
phase observed in many post-stimulus recovery functions,
such a mechanism does not explain the long-term and
permanent stimulus induced changes frequently observed in
lower probe current evoked neural responses. The
mechanism(s) associated with these long-term stimulus
induced changes are probably associated with long-term
metabolic effects.

It has been apparent for some time that there
are two sources of energy associated with neural
activity. First, energy consumed following a single
impulse and restored during the relative refractory
period, and second, a more long-term energy supply which
is more gradually depleted after a prolonged series of
impulses but which also exhibits more gradual recovery
(Forbes and Rice, 1929). It is proposed that significant changes in this second long-term metabolism could result in cell pathology.

Both nerve and muscle make use of normal aerobic metabolic pathways to generate enough energy to ensure their specific physiological function. Glucose is the major substrate associated with this metabolic process (Horowicz and Larrabee, 1958; Hodgkin, 1961; Rarkonen et al., 1969). The energy demands of neural tissue are considerable. Energy is required for a number of cellular processes, some of which are specific to excitable tissue. Cellular maintenance and repair, protein synthesis and axoplasmic transport are included among these energy demanding processes. However, by far the major metabolic demand is associated with the maintenance and restoration of ionic gradients across the neural membrane (Somjen, 1978; Yarowsky and Ingvar, 1981). The energy consumed by this active ionic transport system is dependent on neural spike activity which in turn is dependent on both stimulus intensity and stimulus rate. These observations are consistent with known glucose utilization rates which are quantitatively related to both stimulus rate and intensity (Sokoloff, 1983).

Each spike is associated with a sharp local transient rise in the intracellular sodium concentration ([Na+]i) and the extracellular potassium concentration.
Following propagation of this spike the ionic gradients are rapidly restored to their resting levels by this active transport mechanism, driving sodium - against its concentration gradient - out of the nerve and actively uptaking potassium from the extracellular environment. This so-called "sodium pump" is sodium and potassium specific, obtaining its energy by Na\(^+\)-K\(^+\) ATPase hydrolysis of ATP (Yarowsky and Ingvar, 1981). This pump is actively stimulated by rises in both [Na\(^+\)]\(_i\) and [K\(^+\)]\(_o\) (Somjen, 1978; Galvin et al., 1979). ATP hydrolysed during this activity is regenerated largely by oxidative phosphorylation within the cell mitochondria (Yarowsky and Ingvar, 1981). Greater demands placed on the sodium pump as a result of increased neural activity produces greater metabolic demands on the activated neural population. For example, oxygen (Horowicz and Larrabee, 1958; Ritchie, 1967), glucose (Schwartz et al., 1979; Yarowsky et al., 1983) and ATP utilization (Birnberger et al., 1971) all increase with increasing neural activity.

Although this active process can rapidly restore concentration gradients following the passage of a single pulse, prolonged stimulation produces sustained increases in [Na\(^+\)]\(_i\), [K\(^+\)]\(_o\) and [Ca\(^{2+}\)]\(_i\) (Stockle and Ten Bruggencate, 1980; Galvin et al., 1979; Oritz, 1980). The extent of these stimulus induced changes in local ionic concentrations is dependent on stimulus intensity,
rate and duration (Galvin et al., 1979). Long-term sodium pump activity, as a result of prolonged neural stimulation, places a considerable metabolic demand on the local neural population. In addition the neurone must ensure it has enough energy to satisfy its basic maintenance demands. Excessive stimulus induced demands placed on cell metabolism can lead to a temporary or permanent functional disruption of the cell. As an example, Ochs and Smith (1971), reported temporary stimulus induced reductions in the rate of fast axoplasmic transport in nerve fibres electrically stimulated by short duration current pulses via a monopolar electrode. Significantly, reductions in the rate of fast axoplasmic transport increased with increasing stimulus rate. This transport mechanism provides a means of moving materials manufactured within the soma, such as neurotransmitters and other proteins, to their peripheral sites of utilization. As such, it serves a vital role in cell maintenance, blocking this mechanism can lead to degeneration of the distal neural process (Csillik et al., 1977). Like many of the basic cellular functions, fast axoplasmic transport depends on the same energy source as that which drives the sodium pump (Ochs, 1982). As a second example, a number of investigators have reported large prosodic fluctuations in $[K^+]_o$ during intense electrical stimulation at high rates (Stockle and Ten Bruggencate, 1980; McCreery
and Agnew, 1983). These variations in $[K^+]_o$ were associated with a large reduction in $[Ca^{2+}]_o$ (McCreery and Agnew, 1983). Significantly, these fluctuations were dependent on both stimulus intensity and stimulus rate. This effect ceased when one or the other parameter was reduced. Furthermore, these variations in $[K^+]_o$ corresponded with reductions in the excitability of this neural tract. McCreery and Agnew (1983) interpreted this phenomenon as an inability of the cellular metabolism to maintain local neural homeostasis. There was evidence, at the ultrastructural level, of stimulus-induced damage in nerves stimulated using this intense stimulus (Agnew et al., 1983). Others have also correlated significant increases in $[K^+]_o$ and decreases in $[Ca^{2+}]_o$ with neural pathology (Galvin et al., 1979; Young and Koreh, 1980).

The metabolic demands placed on the auditory nerve fibres during continuous stimulation at high intensities and rates was demonstrated in the present study by the effects of anoxia (Fig. 6.16). In that study the stimulated cochlea was somewhat more vulnerable to the effects of anoxia compared with the unstimulated control cochlea. Moreover, recovery to pre-stimulus levels was slow and not complete.

Not only does prolonged neural stimulation place a considerable metabolic demand on the local neural population, accumulation of these ionic products could
also lead to changes in neural excitability. For example, elevated $[K^+]_o$ will alter spontaneous rates in neurones and reduce the amplitude of the action potential, therefore reducing the efficacy of synaptic transmission (Somjen, 1978; Orkland, 1980). In addition, reductions in $[Ca^{2+}]_o$ as a result of extensive neural activity will effect axon excitability (Nicholson, 1980) and, in the region of the synaptic junction, possibly abolish the post-synaptic response (Miledi and Slater, 1966).

It follows then, that continuous electrical stimulation at high intensities and rates may place significant and even pathological demands on the cell metabolism. These increased energy requirements may compromise the efficacy of the routine metabolic pathways required for normal cellular function. Such changes would significantly reduce neural excitability and could result in cell pathology. Similar metabolic damage models have been proposed to explain cochlear hair cell degeneration following acoustic overstimulation (e.g. Saunders et al., 1985).

It is of interest to discuss the ultrastructural changes observed in the present study in the light of the proposed damage mechanism. In general, spiral ganglion perikaryon examined from cochleas that had been acutely stimulated showed normal ultrastructural morphology. Significantly, in the one case where ultrastructural
damage was observed, the cochlea had been stimulated using high current intensities and rates, which resulted in a permanent stimulus induced reduction in the amplitude of the EABR. These stimulus induced changes in neural excitability were the most extensive observed in the present study.

The cytological changes included severe ballooning and complete disruption of spiral ganglion mitochondria and an apparent reduction in the ribosome content within the cytoplasm. These changes appeared to be localized to ganglion cells in a region close to the stimulating electrodes. Non-excitable tissue in this region (e.g. Schwann cells) showed no evidence of this damage. Moreover, ganglion cells more apical to this site, together with ganglion cells in a similar region of the contralateral control cochlea, also showed no ultrastructural disruption.

The nature of this damage, in particular the widespread disruption of the mitochondria, is consistent with the metabolically based neural damage mechanism implicated in this study. It is conceivable that such a damage mechanism initially manifests itself in the form of damage to organelles whose primary activity is the production of the cells chief energy supply, ATP.

The relationship between the extent of electrically evoked neural activity and ultrastructural changes in the target neural population has also been
observed by others (Agnew et al., 1986). These authors found that selective neural damage occurred following acute stimulation of the cat peroneal nerve. Stimulus induced damage was restricted to the larger diameter fibres within the nerve bundle. Ultrastructural damage, observed one week following the acute stimulus, included the fragmentation of myelin, the formation of myeloid bodies and the loss of axoplasm. As threshold of a nerve fibre is inversely proportional to the fibre's diameter, these results imply that the morphological changes are related to the extent of neural activity.

Since animals in the present study were sacrificed immediately following completion of the acute stimulus regime it is difficult to predict the consequence of the damage observed; i.e. whether or not such damage would ultimately result in ganglion cell loss. However, studies examining more long-term ultrastructural changes, following both acute electrical stimulation (Pudenz et al., 1975a & b; Agnew et al., 1975), and acoustic overstimulation (Rossii et al., 1976; Trevisi et al., 1977), have shown more extensive ultrastructural changes. The extent of these morphological changes increase with post-stimulus survival time. Presumably, the stimulus induced changes observed in the present study are precursors to more extensive neural changes that may result in nerve cell degeneration.
Because the amplitude of evoked responses such as N1E and EABR are dependent on the synchronous activity of a large number of neurones, desynchronization of this activity can result in reductions in the amplitude of the evoked response. It is possible, therefore, that this effect contributed to the stimulus induced reductions in EABR and N1E amplitudes observed in the present study. Considering the rapidity of the short term recovery phase (significant recovery probably occurred during the three second recording period) and the distribution in the neural population contributing to the evoked response, it is likely that a component of the rapid recovery phase was due to desynchronization effects. However, the long-term reductions in evoked response amplitudes were associated with fully recoverable latency functions and would therefore not exhibit effects associated with desynchronization. These long-term reductions in amplitude, monitored for periods of up to 12 hours following completion of the stimulus, are interpreted as a reduction in the population of neurones contributing to the evoked response. Furthermore, since latency functions exhibit complete recovery it may be interpreted that neurones contributing to this reduced response were functioning normally.
There are a number of other points arising from this study that require specific discussion. First, the similar stimulus induced changes observed for both normal hearing and chronic neomycin deafened cochleas implies that the residual ganglion cells, following this pathology, are still capable of normal neural activity in response to electrical stimulation. Therefore, although many of these cells have undergone considerable degenerative changes including loss of dendrites, demyelination of the cell soma and the formation of vacuoles, it would appear that such a neural population can respond normally to the metabolic demands placed on it by repeated electrical stimulation.

Second, it was of some surprise to establish that the origin of the stimulus induced changes in neural activity appeared to lie entirely within the auditory nerve, as the synapse is a readily fatiguable structure (Burke, 1977). The frequency and duration of nerve activation is known to affect the number of neurotransmitter quanta released at the synaptic junction (Rahamimoff, 1974; Robbins, 1974), resulting in a reduction in the endplate potential and therefore reducing the probability of spike generation post-synaptically. This finding implies that the effects of electrical stimulation per se places considerable metabolic demands upon the local neural environment when compared with more remote post-synaptic neurones. The
question as to the site at which these reductions in neural excitability occur is a very important one and one which must be answered before definitive stimulus induced damage mechanisms can be thoroughly understood. It is possible, for example, that while many of these changes are restricted to the proximal neural environment, first order synapses may modify subsequent neural activity thereby reducing the metabolic demands placed on second order neurones. If this were the case then the extent of stimulus induced change observed at the level of the first order neurones would be different to the changes observed post-synaptically due to the modifying behaviour at the synapses. Although this was not observed in the present study, others have reported some variation in activity between pre- and post-synaptic neural pathways following periods of electrical stimulation (McCreery and Agnew, 1983; McCreery et al., 1986).

Finally, the short periods of sensitization observed for low probe current responses following moderate stimulation regimes requires some discussion. Significantly, this effect was not observed following stimulation using high rates and/or high intensities. Moreover, there was evidence of sensitization effects during periods of anoxia. These findings imply that this effect has metabolic origins although the exact mechanisms are quite unclear. Interestingly, sensitization effects are observed in normal cochleas
following periods of relatively intense acoustic stimulation (Hughes and Rosenblith, 1957; Moore, 1968) as well as in the spinal cord, sympathetic motor, visual (Hughes and Rosenblith, 1957) and tactile systems (Moore, 1968). It is not implied that the same mechanism underlie this effect in each pathway, but it would appear to be a common response to periods of moderate neural activity.

There have been a number of reports examining the effects of short periods of electrical stimulation on both the auditory nerve and other neural pathways. Many of these studies were directed at assessing safe levels of electrical stimulation using a variety of neural stimulators (Ignelzi and Nyquist, 1976; Miller et al., 1983; Miller et al., 1985; McCreery et al., 1986). A number of the results reported in the present study have been observed in several of these previous investigations. For example, Pfalz and Pirsig (1966) reported reductions in the CAP evoked from the ipsilateral cochlear nucleus following stimulation with current pulses delivered by electrodes placed on the osseous spiral lamina. The extent of reduction in amplitude of the CAP increased with stimulus rate. Moreover, these evoked potentials were considered to be metabolically active as they were readily reduced in the presence of anoxia and restored following restoration of the oxygen supply. This finding supports the present
observation that these stimulus induced changes are of metabolic origin.

Prijs (1980) recorded the electrically evoked N1 from recording electrodes placed at both the round window and the internal auditory meatus. These N1E's were evoked by orthodromic and antidromic stimulation using short duration current pulses. Reductions in N1E amplitude was a function of stimulus rate with significant reductions only occurring for rates in excess of 100 pps. Since the electrical stimulus effectively bypassed the hair cell - auditory neurone synapse, Prijs interpreted these changes as being associated with the auditory nerve per se. This was confirmed following similar results in response to antidromic stimulation from electrodes placed on the VIIIth nerve within the internal meatus. These changes were interpreted as neural fatigue induced by repeated stimulation.

Miller and his colleagues (Miller et al., 1983; Miller et al., 1985) reported reversible increases in EABR thresholds following 3 hour periods of monopolar stimulation using continuous 1 kHz sinusoidal electrical stimuli. These elevated EABR thresholds, recorded just following completion of the acute stimulation period, fully recovered prior to a subsequent stimulation session one week later. Concurrent with threshold changes were reductions in amplitude and increases in latency of the post-stimulus EABR. Consistent with the duty cycle

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results in the present study, these investigators noted that intermittent stimulation (in contrast to the continuous stimulation normally used) resulted in reductions in the degree of stimulus induced changes in the EABR. Interestingly, the extent of these stimulus induced changes varied with site of stimulation. The most extensive changes were associated with electrodes located within the scala tympani. Electrodes placed either on the round window or promontory produced progressively less stimulus induced changes for stimulation at the same intensity. These results can be interpreted as being due to differences in stimulus intensity at the site of the neural tissue as electrodes placed within the scala tympani are much more effective in evoking auditory nerve activity than electrodes placed on the round window or otic capsule (Shepherd and Clark, unpublished observations; Simmons et al., 1985). These results would therefore appear to be consistent with the results obtained in the present study where stimulus intensity correlated with the degree of stimulus induced change in the EABR.

Changes in the excitability of neural populations in response to acute periods of electrical stimulation have also been reported by investigators studying other neural pathways. For example Zigmond and Chalazonitis (1979) reported frequency dependent reductions in the amplitude of the CAP evoked from the
rat preganglionic cervical sympathetic trunk in response to pulsatile electrical stimulation. Significant reductions in the amplitude of the CAP were reported following stimulation at frequencies of 16 and 20 pps. Above this rate the CAP disappeared within seconds of stimulus onset. In addition, these investigators reported a reduction in the extent of stimulus induced change in the CAP following stimulation using a duty cycle. These results are qualitatively similar to the results obtained in the present study and support the contention that these stimulus induced changes are dependent on the extent of neural activity. Similar stimulus induced changes have been reported following stimulation of the cat sural nerve at rates of 10-15 pps (Ignelzi and Nyquist, 1976).

The significant differences in stimulus rates at which these changes occur presumably reflect the large variations in metabolic capacity of the neural pathways within the CNS. These differences in metabolic capacity reflect underlying functional differences between these pathways. For example, neurones within the preganglionic cervical sympathetic trunk normally fire at extremely low rates (i.e. 1-2 pps; Zigmond and Chalazonitis, 1979). It is not surprising then, that these neurones cannot sustain firing rates of 30-50 pps. In contrast, results from the present study
demonstrated that the auditory nerve can follow stimulus rates of 100-200 pps with little reduction in excitability. Only at higher stimulus rates were significant changes in excitability observed. In normal cochleas, the auditory nerve can be expected to fire at rates of up to 200-300 spikes per second in response to acoustic stimuli. Indeed the auditory nerve is capable of higher sustained firing rates than any other mammalian nerve (Hodgkin, 1961). These observations presumably reflect the enhanced metabolic capacity of the auditory pathway when compared with other neural pathways. It is therefore not surprising that the auditory brainstem has the highest glucose consumption rate of any neural pathway within the mammalian brain (Sokoloff, 1983).

A number of other investigators have also noted that significant stimulus induced changes in neural excitability occur only at stimulus rates somewhat above rates experienced naturally for that particular neural pathway (Yarowsky et al., 1983; McCreery et al., 1986). If these observations are correct then a thorough understanding of the neural response patterns to natural stimuli are required prior to the design of stimulation protocols for neural prostheses. Moreover, these stimulus protocols should evoke neural activity that closely mimics the firing patterns occurring naturally.
Because the inner spiral fibres of the cochlear efferent system are in synaptic contact with the primary cochlear afferents (Spoendlin, 1966, 1969), they are in a position to modify afferent activity. There is some evidence that monopolar electrical stimulation using a round window electrode could alter acoustically generated N1 responses by electrically stimulating efferent fibres (Rajan and Johnstone, 1983). It was therefore considered necessary to determine whether this efferent pathway contributed to the stimulus induced changes observed in the present study. The lack of effects of systemic administration of strychnine, a potent efferent blocking agent (Bobbin and Konishi, 1974; Klinke and Galley, 1974) on these stimulus induced changes indicated that the efferent pathway played no part in these changes. This finding was not altogether surprising. While synaptic contact between the efferent pathway and the primary auditory afferents innervating the inner hair cell occurs immediately below the inner hair cells, the site of generation of action potentials within these afferent fibres, as a result of electrical stimulation, would occur at nodes of Ranvier located more centrally than this. Increasing the stimulus intensity would move the site still more central to this synaptic junction. Under these circumstances peripherally located efferents could not influence this activity.
Although anoxia studies performed in the present research indicated that electrically stimulated cochleas were more metabolically vulnerable than unstimulated control cochleas, latter waves in the EABR consistently exhibited significantly greater anoxia induced reductions in amplitude compared with earlier waves. This observation was observed for both stimulated and control cochleas. An example of the effects of anoxia on the EABR waveform is illustrated in Fig. 6.29. In this example the cochlea was stimulated at a stimulus intensity of 1.5 mA, 200 μs per phase at a rate of 200 pps. The initial response (0 min) was recorded just prior to the start of the stimulus period. The following four responses (19, 22, 24 and 26 min) were recorded during the acute stimulus period. The animal was made anoxic 10.5 minutes following commencement of stimulation by ventilating it for 10 minutes with 100% nitrogen. Following this 10 minute period the animal was ventilated with 100% oxygen. The significant effects of anoxia are apparent. The maximum reduction in EABR waveform (22 min) exhibited almost complete loss of waves III and IV. Significantly, earlier waves were less affected. Recovery from anoxia (24 and 26 min) exhibited increased amplitudes and decreased latencies for all waves of the EABR. The mechanism underlying these differential changes in response to anoxia is unclear. One possibility is that latter waves originate
from generators more metabolically active than the
generators of earlier waves. Some support for this
hypothesis comes from the fact that the inferior
colliculus - a significant contributor to the generation
of these latter EARR waves - has the highest consumption
of glucose of any structure within the mammalian CNS
(Sokoloff, 1983). Alternatively, these changes may
reflect vulnerability of synaptic structures under
conditions of anoxia.

There are a number of similarities between the
stimulus induced changes reported in the present study
and stimulus induced changes following acoustic
stimulation in the normal cochlea. For example, the two
phased recovery process observed following electrical
stimulation is qualitatively similar to the recovery
process observed following long-term acoustic stimulation
(Peake et al., 1962; Wilpizeski and Tri, 1964, Ward,
1973). In both cases the extent of these stimulus
induced changes are dependent on stimulus rate, stimulus
intensity, stimulus duration and duty cycle. The short-
term recovery process associated with acoustic
stimulation is observed in the N1 while cochlear
microphonics remain unaltered. These rapid changes
therefore appear to be central to the hair cells and have
been at least partially attributed to the refractoriness
within the auditory nerve (Eggermont, 1985). It is
therefore likely that these short-term changes share a
common mechanism with the short-term changes associated with electrical stimulation of the auditory nerve.

More long-term reductions in sensitivity are termed auditory fatigue or temporary threshold shift (TTS). The site of these long-term changes appears to be associated with the sensory hair cells and their synaptic junction with the primary cochlear afferents (Robertson, 1983; Saunders et al., 1985). Like the long-term changes induced with electrical stimulation, TTS recovers gradually (over minutes or hours) and recovery is dependent on the size of the initial TTS (Elliott and Fraser, 1970). Furthermore, if the stimulus is intense enough or of sufficient duration these reductions in auditory sensitivity can be permanent resulting in pathological changes at the level of the hair cell. Depending on the nature of the stimulus, these pathological changes are associated with either mechanical or metabolic insult (Saunders et al., 1985).

6.6 **Summary.**

The present results have demonstrated that acute electrical stimulation, using short duration biphasic stimuli can result in both short-term and permanent reductions in excitability of the auditory nerve. These stimulus induced changes included a reduction in the amplitude and an increase in both the latency and threshold of the electrically evoked response.
Figure 6.29 A series of EABRs recorded prior (0) and during (19, 22, 24 & 26 min) intracochlear electrical stimulation at 1.5 mA, 200 us per phase and 200 pps. A 10 minute period of anoxia commenced 10.5 minutes following the start of stimulation. The animal was respired with oxygen after completion of the anoxic period. Note the greater susceptibility of latter waves in the EABR to anoxia.
These stimulus induced changes in excitability were dependent on the extent of stimulus induced neural activity. Greater stimulus induced changes were associated with stimulus regimes having high stimulus intensities and rates. While stimulus duration also contributed to these changes its effect was somewhat less than intensity or rate. Using a stimulus regime with a duty cycle of less than 100% also reduced the extent of these changes.

These stimulus induced changes were dependent on the spatial relationship between the bipolar electrode array and the auditory nerve fibres. Low threshold neural populations, which were therefore close to the stimulating electrodes, exhibited greater stimulus induced changes than populations more remote from the electrode pair.

The mechanisms underlying these stimulus induced changes were attributed to short and more long-term metabolic changes in the excited neural population. Permanent stimulus induced changes, observed in one cochlea in the present study, presumably reflects the cells inability to maintain its basic homeostatic mechanisms as a result of the extensive metabolic demand placed on it by the highly deterministic nature of the stimulus. Ultrastructural examination of ganglion cells in this cochlea revealed disruption to mitochondria in cells located in the basal turn close to the stimulating
electrodes. Ganglion cells more remote from the electrode array, or from the contralateral control cochlea, did not exhibit these morphological changes.

These results have, therefore, shown that stimulus parameters other than charge density must be considered when assessing neural damage mechanisms associated with neural stimulators. Moreover, the present research has provided some insight into the response of neural tissue to unnatural stimuli such as an electric pulse. An understanding of the mechanisms associated with these stimulus induced changes will lead to an increased knowledge of the functional mechanisms and limitations associated with artificial electrical stimulation of neural tissue.
CHAPTER SEVEN

CONCLUDING REMARKS AND RECOMMENDATIONS FOR FUTURE RESEARCH.

The biocompatible nature of long-term intracochlear electrical stimulation using short-duration charge balanced biphasic current pulses and Pt stimulating electrodes, has been demonstrated in the present research. In addition, this work has also shown that the in vivo stimulated Pt electrodes are not subjected to excessive electrochemical stresses that would result in significant Pt dissolution. Finally, evidence of both temporary and permanent reductions in the excitability of the auditory nerve was observed following acute stimulation at intensities and rates significantly above the levels being used clinically. These findings implicate a metabolic model as a neural damage mechanism associated with electrical stimulation.

There are a number of questions that arise out of the present investigations that should be pursued in future research.

The results of the chronic stimulation study (Chapter 4) illustrated the adverse effects of infection associated with cochlear prostheses. Future investigations designed to examine techniques that will minimize the risks of infection are required. These investigations should include the development of
strict surgical protocols to reduce the chance of infection, and the use of appropriate biomaterials to ensure the development of an effective seal at the implanted round window. These sealing techniques should be evaluated in suitable animal models.

The precursor to new bone growth within the scala tympani remains unclear as does the period over which osteogenisis remains active. A consistent finding in the present study was the formation of new bone at the endosteum in response to a generalized fibrous tissue reaction. Valuable future studies would therefore include an investigation of biomaterials and surgical techniques designed to minimize the fibrous tissue reaction. In addition, the initial site of new bone growth and its development with time, could be examined with the use of fluroescein dyes. These dyes become incorporated into the matrix of the new bone. Their injection at various periods following electrode implantation could provide valuable information regarding the time course of osteogenisis within the cochlea.

It is conceivable that damage to the basilar membrane does not result in localized extensive nerve fibre loss in profoundly deafened animal models. To date all investigations of this kind have used normal hearing animals. In profoundly deaf preparations it is possible that tears localized to the basilar membrane may not result in extensive neural loss as there would be few
dendrites projecting to the level of the basilar membrane. The histopathological consequence of damage to the basilar membrane, together with other cochlear structures including Reissner's membrane and the spiral ligament, need to be investigated in an appropriate animal model.

The present study illustrated a qualitative relationship between cochlear histopathology and the EABR. It remains to be determined whether the EABR can be used to provide a more quantitative estimate of auditory nerve survival. A study examining such a relationship could result in a useful clinical test for screening potential cochlear prosthesis candidates. Such a study should examine EABRs, evoked by a variety of monopolar and bipolar electrode geometries, in animals with a wide range of surviving auditory nerve fibres.

In addition to these proposals, there is a need to compliment the present investigation with an extensive ultrastructural examination of ganglion cells subjected to long-term electrical stimulation. There is also a need to perform extensive physiological and histopathological studies on the effects of long-term electrical stimulation in the maturing auditory pathway in young animals both with and without prior auditory experience.

Finally, new stimulation regimes are being developed in order to provide more sophisticated
stimulation strategies. A thorough examination of the safety and efficacy of these new stimulation regimes - including their effects on the Pt stimulating electrodes - is required prior to their clinical application.

Many of the in vivo stimulated electrodes in the present research exhibited significant short-term fluctuations in electrode impedance (Chapter 5). It has been suggested that these short-term changes in impedance are associated with the formation of a protein film overlying the electrode. Appropriate electrochemical studies investigating these mechanisms could result in techniques that would minimize these impedance changes. Although these fluctuations in impedance do not, in general, cause a difficulty in the ability of the stimulator to inject constant charge, these changes could result in a short-term loss of voltage compliance in situations where the baseline electrode impedance is high. Loss of voltage compliance is undesirable as it could result in stimulation using unbalanced current pulses.

Metabolic changes in the auditory nerve were implicated as the underlying physiological mechanism associated with stimulus induced reductions in the excitability of the auditory nerve (Chapter 6). This proposed model could be evaluated by performing biochemical and metabolic studies of the electrically stimulated auditory nerve. Ion-sensitive
microelectrodes could be used to monitor \([K^+]_o\), \([Na^+]_o\), \([Ca^{2+}]_o\) and \([O^{2-}]_o\) during rest and periods of acute electrical stimulation. Moreover, histochemical techniques could be used to examine the ATP content of both control and stimulated auditory nerve fibres. Such data, obtained for a variety of stimulus regimes, would provide an insight into the metabolic response of the auditory nerve to acute electrical stimulation and would help establish over what stimulation range the auditory nerve can maintain homeostasis.

An extension of this work would be an examination of the metabolic response of electrically stimulated auditory nerve fibres from both normal hearing and chronically deafened animal models. This work could also be extended to evaluate new stimulation regimes.

Finally, the mechanisms underlying "tone decay", observed in many implant patients in response to short periods of continuous electrical stimulation, could be investigated in light of the per-stimulus changes in excitability observed in the present study. The great variability of "tone decay" among implant patients is intriguing. Since many patients that exhibit extensive "tone decay" are poorer prosthesis users, knowledge of the mechanism(s) responsible for this effect may result in the development of screening tests that give an indication of the likely performance of patients.
APPENDIX I

ABR/EABR STIMULUS AND RECORDING CIRCUITRY.

Brainstem Response Signal Averaging Block Diagram
APPENDIX II

DC BIAS CURRENT MEASUREMENT OF PORTABLE CURRENT STIMULATORS.

Charge Imbalance Measurement Circuit

Typical Charge Imbalance for Portable Cat Stimulators

Stimulator current (mA)

DC Imbalance (μA)

AII-1
APPENDIX III

HISTOLOGICAL SCHEDULES.

PROCESSING CAT COCHLEAS FOR LIGHT AND ELECTRON MICROSCOPIC STUDIES.

A. Perfusion solutions:

0.2 M phosphate buffer pH 7.3 - 7.4
25% glutaraldehyde solution (EM Grade)
5% paraformaldehyde solution (EM Grade)
5% calcium chloride solution
1% osmium tetroxide
4% EDTA in trimming fluid

Pre-perfusion fluid:
Make up 500 ml solution of distilled water containing:

9.0 g NaCl
10 ml calcium chloride solution
100 ml sucrose solution

N.B. calcium chloride must be added slowly with continuous stirring at room temperature to avoid a precipitate forming.
Add 500 ml of phosphate buffer and adjust to pH 7.35. Just prior to perfusion, 3 ml of 10% sodium nitrate solution and 0.2 ml/kg body weight heparin must be added to this solution. This solution should be used at 37°C.

Perfusion fluid:
Make up 500 ml solution of;

200 ml paraformaldehyde
40 ml glutaraldehyde solution
10 ml calcium chloride solution
250 ml distilled water

Add 500 ml phosphate buffer and adjust pH to 7.35. This solution should be used at 37°C.

Trimming fluid:
Make up a 11 solution of;

200 ml glutaraldehyde solution
200 ml sucrose solution
20 ml calcium chloride solution
580 ml distilled water

Make up to 11 with phosphate buffer and adjust to pH 7.35. This solution should be used at 4°C.
B. Post-perfusion tissue preparation:

Following systemic perfusion the temporal bones are removed from the skull and excess tissue removed. The oval window is opened and a small hole is placed in the otic capsule overlying the apical turn. This procedure should be performed while the bone is immersed in cold trimming fluid. The cochlea is then placed in clean, cold trimming solution overnight. The otic capsule is thinned by drilling the cochlea under cold trimming fluid. This fluid must be changed frequently to avoid histological artefacts.

All subsequent steps are performed at 4°C on the specimen rotator until the cochleas are placed in acetone.

After thinning the otic capsule the cochleas are placed in EDTA fluid until decalcification is complete as determined by x-ray. The EDTA fluid is changed every second day.

Following completion of decalcification the cochleas are washed in several changes of trimming solution over a 36 hour period to ensure removal of the EDTA.

The cochleas are then rinsed in phosphate buffer containing 0.05 M sucrose and 0.005% calcium chloride for three changes of one hour each.
Secondary fixation is achieved by placing the cochlea in 1% osmium tetroxide solution for one hour. Following osmification the cochleas are washed in 0.1 M phosphate buffer containing 0.05 M sucrose and 0.005% calcium chloride for three changes of 10 minutes each.

The cochleas are then dehydrated and infiltrated according to the following schedule:

- distilled water 2 x 10 minutes
- 30% alcohol 2 x 15 minutes
- 70% alcohol with saturated uranyl acetate overnight
- 90% alcohol 1 x 15 minutes

Place at Room Temperature

- 100% acetone 3 x 15 minutes
- 1:1 Spurr’s resin and acetone 2 hours
- 3:1 Spurr’s resin and acetone overnight
- 100% Spurr’s resin 2 x 4 hours
- Polymerise at 80°C overnight.


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