Title

Chronic electrical stimulation by a cochlear implant prevents degeneration of spiral ganglion neurons and induces temporal and spatial plasticity in the mature deafened auditory system

Author

Moore, Charlotte M.

Publication Date

1999

Peer reviewed|Thesis/dissertation

CHRONIC ELECTRICAL STIMULATION BY A COCHLEAR IMPLANT PREVENTS DEGENERATION OF SPIRAL GANGLION NEURONS AND INDUCES TEMPORAL AND SPATIAL PLASTICITY IN THE MATURE DEAFENED AUDITORY SYSTEM

by

Charlotte M. Moore

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

SPEECH AND HEARING SCIENCE

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

Dedicated to my family.

Acknowledgements

I would like to thank Dr. Patricia Leake. Her drive and dedication to the field of cochlear implants has been inspiring.

Thanks to everyone in the Epstein Laboratory. Specifically, Russell Snyder, Steve Rebscher, Gary Hradek, Maike Vollmer and Beth Dwan for their assistance in every facet of this project.

Charlotte M. Moore

ABSTRACT

Previous studies have shown that chronic intracochlear electrical stimulation (ICES) delivered by a cochlear implant alters physiological response properties of neurons in the auditory midbrain and prevents degeneration of spiral ganglion cells (SGC) in the *developing* mammalian auditory system. In order to investigate the effects of chronic ICES on the *mature* auditory system, the spatial and temporal representations of electrical signals in the central nervous system and SGC survival were examined in adult deafened, chronically stimulated animals.

Adult cats were deafened by ototoxic drugs and unilaterally implanted with intracochlear electrodes. Animals were stimulated with electrical pulse trains (300 pulses/second amplitude modulated at 30 Hz) for 4 hr/day for 4-6 months. Electrically evoked auditory brainstem responses (EABR) to increasing electrical pulse rates were examined before and after chronic stimulation. In final electrophysiology experiments single neuron responses to pulse trains of increasing frequency were recorded from the contralateral inferior colliculus (IC). Maximum frequency following (Fmax) and onset latencies were determined separately for the external (ICE) and central (ICC) nuclei. Spatial (frequency) selectivity was evaluated by plotting threshold vs depth functions (spatial tuning curves; STC) and measuring the 6dB width. Finally, the cochleae were evaluated histologically and spiral ganglion cell survival was assessed.

EABR amplitude decreased with increasing electrical stimulus pulse rates in all animals. In addition, the frequency at which the amplitude decreased by half was greater following chronic electrical stimulation. Temporal resolution of ICE neurons

in stimulated animals was not significantly different from ICE neurons recorded from normal animals. However, ICC neurons displayed significantly ($p<0.001$) higher temporal resolution. Mean Fmax was higher (137 pps) and latency was shorter (5.8 ms) than in normals (102 pps; 6.9 ms). These results were nearly identical to results in neonatally deafened chronically stimulated animals. In addition, the spatial resolution of the ICC was altered by ICES. STC widths were considerably wider (1.1 mm) than those reported for normals (0.64 mm) but not as broad as those reported in neonatally deafened and chronically stimulated animals. Finally, although SGC density was reduced bilaterally, the mean overall SGC density in stimulated cochleae was 10% greater than in unstimulated cochleae.

In summary, these results indicate that the *adult* deafened auditory system is significantly altered by chronic electrical stimulation in ways similar to changes seen in neonatally deafened, chronically stimulated animals. Chronic electrical stimulation of the *adult* animal has a protective effect on SGC survival, increases temporal resolution and decreases spatial (frequency) selectivity. Taken together, these results suggest that chronic stimulation by a cochlear implant induces marked anatomical and physiological changes, reflecting substantial plasticity in the mature auditory system.

Table of Contents

Page Number

List of Tables

Page Number

List of Figures

1.0 INTRODUCTION

For three decades cochlear implants have been used to restore auditory input to profoundly deaf adults and children who obtain minimal or no benefit from conventional hearing aids. Since substantial benefit has been obtained by these groups, the application of implants has been extended to adults who demonstrate some benefit from hearing aids (NIH, 1995). The benefits from these devices have been characterized by wide variability in performance among users. Some individuals show remarkable benefit and are able to use a conventional telephone almost immediately, whereas others receive only minimal benefit and show poor speech discrimination even after long periods of use. These extreme differences have been attributed to a number of factors including age at onset, duration and etiology of deafness, placement of the intracochlear electrodes and functional integrity of the central auditory system.

Age at onset of deafness has been shown to contribute significantly to performance with a cochlear implant (Staller et al., 1991). Individuals deafened prior to learning speech and language (congenital or very early onset of deafness) typically do not perform as well as those with an onset of deafness during or following the acquisition of speech and language. Therefore, the later a child becomes deaf (i.e., the more exposure to speech and language), the better their performance with a cochlear implant (Osberger et al., 1991). This finding has been attributed to a 'critical period', a period of time during development in which auditory input is necessary for the normal acquisition of speech and language (Rubens and Rapin, 1980; Eggermont and Bock, 1986). The widespread belief in

the importance of this critical period has provided the rationale for the implantation of children as young as 18 months of age.

Further support for implanting very young children comes from the assumption that the young nervous system is more malleable (plastic) than the mature auditory system and, therefore, has a greater capacity of adaptability to interpret the highly artificial electrical signals presented through a cochlear implant. However, significant plasticity also has been observed in the mature nervous system (e.g. see Kaas, 1997). In fact, adults deafened for long periods of time and then implanted with cochlear implants often obtain substantial benefit, but this benefit may require a long period of use before significant improvement is observed and/or before performance plateaus (Schindler et al., 1995). The improvement over time and the ability to adapt to the aberrant electrical signal suggests that the mature auditory system is capable of significant plasticity.

While much is known about the effects of deafness on the anatomy of both the developing and mature central auditory system, far less is known about the effects of chronic electrical stimulation. In animal models of congenital and early acquired deafness, chronic intracochlear electrical stimulation has been shown to decrease and/or prevent some of the deleterious effects of deafness on the auditory system. For example, studies have suggested that ICES can delay or partially prevent the retrograde degeneration of the primary auditory neurons (Leake et al., 1991; 1992), broaden the central representation of the chronically stimulated sector of the cochlea (Snyder et al., 1990; 1991) and increase the frequency following

capabilities of central auditory neurons (Snyder et al., 1995; Vollmer et al., In press).

While these findings are significant for the *early* deafened animal, the role of development in mediating these effects has not been determined. Although some studies in *adult* animals have suggested that spiral ganglion degeneration after deafness is ameliorated by chronic stimulation (Lousteau 1987; Hartshorn et al., 1991), other investigations have failed to demonstrate these effects (Shepherd et al., 1994; Araki et al., 1998; Li et al., 1997; In press). Moreover, studies of the functional consequences of electrical stimulation in inducing plasticity of the *adult* central auditory system have not been reported. Therefore, the object of the present study was to investigate the effects of intracochlear electrical stimulation on the mature auditory system. Such a study is necessary to examine the effects of electrical stimulation without the influence of critical periods of development. The effects of electrical stimulation on the central auditory system are especially important to understand and consider as an increasing number of people are being considered for cochlear implantation. Further, since current cochlear implant users show such great variability of results, examination of the effects of electrical stimulation on the mature auditory system is an important step in providing a more comprehensive understanding of the anatomical and functional changes occurring in the auditory system of cochlear implant users.

1.1 Sensory Deprivation

In general it is agreed that the severity of effects of sensory deprivation is related to the age at which the deprivation occurs. Studies of the auditory, visual and somatosensory systems have provided evidence that the earlier the loss of sensory input, the more devastating the effects (for review see Kaas, 1991; Kaas, 1997). Both morphological and physiological studies have demonstrated that immature animals are more vulnerable to sensory deprivation than mature animals. Further, they have suggested that a 'critical period' of time exists during which sensory input is essential to normal maturation and development.

In the auditory system a number of morphological studies have compared the effects of auditory deprivation in developing and mature animals. In general, the studies have found that young deafened animals sustain greater and more rapid changes in auditory brainstem neurons (e.g., loss and shrinkage of cells and neuropil) than older deafened animals (Webster and Webster, 1977; Trune, 1983; Born and Rubel, 1985; Moore and Kowalchuk, 1988; Hashisaki and Rubel, 1989). As an example, chinchillas and gerbils deafened early in life have smaller ventral cochlear nucleus neurons than adult deafened and control animals (Hashisaki and Rubel, 1989; Fleickeisen et al., 1991).

The resilience of the adult system to change may be related to critical periods of early development such that once normal connections are established they are not easily altered. Knudsen (1998) demonstrated that owls with altered juvenile auditory experience had a greater capacity for plastic changes later in life. These data suggest that experience during a 'sensitive period' sets up long

lasting functional connections which may be referenced in adulthood. Taken together, these results suggest that the young developing nervous system may require input for establishment and maintenance of organization, whereas mature animals may have 'established connections' which may not require constant input for maintenance.

This is not to say that the adult system is completely unaffected by deprivation of input. Although the effects of neonatal auditory deprivation may be much more severe, many morphological and physiological studies have reported changes in the adult nervous system with deprivation. Retrocochlear degeneration in first order neurons as well as transneuronal changes in central auditory nuclei at all levels up to the inferior colliclus have been observed in animals deprived as adults (Spoendlin, 1975; Jean-Baptiste and Morest, 1975; McGee and Olzewski, 1962).

Electrophysiological mapping studies have been used to study various aspects of the functional organization of the deprived central auditory system. Shirane and Harrison (1991) reported a significant difference in the responses recorded from neonatally deafened and adult deafened animals. They reported that inferior colliculus (IC) neurons were essentially unresponsive to cochlear electrical stimulation in animals that were deaf from birth, and responses were severely reduced in adult deafened controls as well. Contrary to this report, Snyder et al. (1991) found that temporal properties of IC neurons in response to intracochlear electrical stimulation were similar in neonatally deafened and adult deafened animals that had **no** prior history of electrical stimulation. Moreover,

despite the fact that the neonatally deafened animals had no normal auditory experience, central tonotopic organization was established and maintained and was, in fact, similar to adult animals following a lifetime of auditory experience. This finding suggested that at least some aspects of functional organization are innate and do not require input for establishment, or at least, are accomplished before opening of the external ear canal.

1.2 Plasticity

A number of studies have shown that dramatic changes within the central nervous system can be forced by altering peripheral input. In general, the animals in these studies were deprived or received altered sensory input. The effects were then measured by using behavioral, morphological and/or electrophysiological methods. Most studies demonstrated that a change in sensory input results in profound and long-lasting alterations within the central nervous system. The ability of the nervous system to make such adjustments to sensory inputs has been called plasticity (For review see Buonomano and Merzenich, 1998).

Plasticity was initially demonstrated in cortical regions. However, more recent studies have confirmed that plasticity occurs in most sensory systems in subcortical areas as well. These morphological, physiological and perceptual alterations have been observed in studies of the visual, motor, somatosensory and auditory systems.

Depriving or altering the visual input to animals by removing or occluding one or both eyes, creating retinal lesions, or artificially stimulating the eye can

create functional, as well as morphological changes within visual cortex (For review see Movshon and VanSlyters, 1981). In such experiments primary visual cortex of adult mammals can remain altered for a short or extended period of time. Small retinal lesions result in an alteration of receptive fields, which in turn alter the topographic arrangement of cortical maps. Cortical regions originally responsive to the area of peripheral lesion acquire receptive fields, which are responsive to the inputs from the surrounding unlesioned area of the retina (Chino et al., 1992). Similar results have been described in studies using artificial scotomas and conditioning stimuli to mimick lesion studies. Continuous stimulation with random flashing light around a hole masking the receptive field region produces a large expansion of the receptive field. Further, this increased receptive field size can be reversed by stimulation of the original receptive field. (Volchan and Gilbert, 1995). Additionally, somewhat indirect evidence for visual system plasticity has been observed in humans. Subjects receiving extensive visual training are capable of substantially improving performance on tasks of motion discrimination, pattern recognition and orientation (Ball et al., 1982; Crist et al., 1997; Fahle 1998). Kapadia et al. (1994) and Crist et al. (1997) created artificial scotomas in humans by presenting a masked area with a surrounding changing visual pattern. The subjects' ability to spatially orient short lines was biased towards the interior of the scotoma. The authors attributed this misalignment of the stimulus position to the receptive field expansions observed in similar animal physiological studies.

The authors of these visual system studies have suggested that the observed changes have been brought about by an increase in the effectiveness of already existing subthreshold connections. A decrease in intracortical inhibition brought about by the conditioning techniques may unmask the normally subthreshold excitatory inputs (Chapman and Stone, 1996) resulting in the expanded receptive fields observed in the animals and the misalignment observed in psychophysical studies.

Plasticity within somatosensory cortex and brainstem regions has been documented by removing or altering peripheral input (For review see Merzenich, 1984; Wall and Kaas, 1986; Xu and Wall, 1997). Crushing or sectioning a nerve deprives the brainstem and cortex of normal input and results in an expansion of somatosensory areas from the lesion edge. In adult rats, cutting some whiskers and sparing others biases the input to the somatosensory system (Melzer and Smith, 1998). A decrease in the efficiency of response to the trimmed whiskers can be observed by recording the responses of cortical cells to stimulation of the whiskers. Areas of barrel cortex are expanded to the 'biased' input whiskers and much reduced in response to stimulation of the cut vibrissae.

Analogous experiments also have been conducted in the auditory system. The tonotopic organization of the central auditory system is altered after the cochlea is damaged (e.g. Robertson and Irvine, 1989; 1993; Harrison et al., 1993; Willott et al., 1993; Schwaber et al., 1993; Snyder and Sinex, 1998). Robertson and Irvine (1993) found that partial cochlear damage is reflected in marked changes in the cochleotopic organization of the auditory cortex of the

guinea pig. Discrete high frequency cochlear lesions resulted in areas of cortex that were originally most sensitive to the lesioned cochlear frequency range becoming responsive to adjacent unaffected frequencies. Schwaber et al. (1993) found similar reorganization in the auditory cortex of monkeys following hearing loss induced by ototoxic drugs. Although only three animals were studied, cortical maps examined before and after ototoxic deafening were consistent with extensive reorganization of primary auditory cortex following drug administration.

Evidence for reorganization also has been observed in the inferior colliculus (Moore and Irvine, 1981; Willott, 1986; Harrison et al., 1993; Irvine and Rajan, 1994; Wang et al., 1996) and cochlear nucleus (Willott et al., 1991; Kaltenbach et al., 1992) following peripheral deprivation or insult. Moore and Irvine (1981) produced unilateral conductive hearing losses by ligation of the external auditory meatus of neonatal and adult animals. They compared the thresholds, response strength to contralateral stimulation, and numbers of interaural intensity sensitive units for the ligated animals and a group of normal (unligated) animals. Thresholds and mean firing rates of neurons to contralateral stimulation were not significantly different between the ligated and unligated animals. However, a reduction in the number of interaural intensity difference sensitive neurons was found in the ligated animals, both adults and neonates. The authors suggest that this result was due to changes during the ligation period in which the nervous system was modified to maintain a balance between the two ears. Thus, ligation decreases the input, and the effectiveness of ipsilateral input is then somehow

reduced in order to maintain a balance between the (excitatory and inhibitory input) two ears for both the adult and neonatal animals.

Evoked potential recordings also have provided evidence for plasticity in the central auditory system. Changes in the amplitude of evoked potentials have been reported following altered cochlear input. Gerken et al. (1984) implanted stimulating electrodes in the cochlear nuclei and inferior colliculi of adult cats. After obtaining baseline behavioral thresholds and evoked potential responses to electrical signals, mild to moderate hearing losses were induced by high intensity noise exposure. Subsequent testing revealed a mean *decrease* in electrical thresholds and an *increase* in evoked potential amplitude despite the peripheral damage. Salvi et al. (1990) observed an increase in evoked potential response amplitude when a high frequency sensorineural hearing loss was induced at the auditory periphery. The increased amplitude occurred for frequencies on the edge of the lowest frequency of the noise induced hearing loss. This suggested that the amplitude was increased due to a loss of inhibition from the high frequency (hearing loss) regions. Additional evoked potential studies have reported similar findings and have supported this hypothesis of decreased inhibition (Gerkin et al., 1984; Wang et al., 1996; Szczepaniak and Møller, 1996).

Experience and learning also have been shown to induce changes in the central auditory nervous system (For review see Weinberger, 1997). Bakin and Weinberger (1990) implanted microelectrodes into the auditory cortex of guinea pigs and then evaluated the frequency receptive fields before and after classic conditioning. They presented tones paired with mild electric shocks to the foot.

After this conditioning the receptive field was shifted away from pretrained frequencies towards conditioned frequencies.

Recanzone et al. (1993) trained monkeys to discriminate tones of different frequencies, then examined the frequency organization of the auditory cortex using electrophysiological techniques. The results revealed an expansion of the cortical area representing the trained stimuli. The expanded representations were two to eight times greater than those of normal controls.

Similar alterations have been observed (without training) in studies pairing tones with stimulation of the nucleus basalis (Bakin and Weinberger, 1993, 1996; Kilgaard and Merzenich, 1998). These studies have shown that the pairing of auditory stimuli with electrical stimulation (shocks) to the nucleus basalis produces a reorganization of the primary auditory cortex in adult animals. In addition, it was shown that the auditory receptive field sizes could be altered depending on the specific parameters of the acoustic stimulus.

In summary, in contrast to the classical concept that the mature nervous system is largely 'hard-wired' and relatively incapable of plasticity, significant alterations have been demonstrated in the mature mammalian central nervous system. Further, contrary to earlier views, changes observed in subcortical regions are as profound as those observed in the cortex.

1.3 Electrical Stimulation

The major goal of neuroprosthesis research has been to provide or reestablish input to damaged sensory systems. In this attempt, electrical stimulation has been

applied to re-activate the visual, auditory, sensory and motor pathways. Studies of the effects of this aberrant stimulation have shown that electrical stimulation may in fact effectively provide input, however, in doing so neural connectivity and physiological response properties of the nervous system may be altered.

Electrical stimulation of a peripheral sensory nerve produces an expansion of the somatosensory receptive field for that nerve in the cortex of adult animals (Recanzone et al., 1990). For example, chronic stimulation of the median nerve expanded the region of somatosensory cortex responsive to the median nerve. Similarly, intracortical electrical stimulation of the adult monkey primary visual areas resulted in synaptic plasticity which varied depending on the cortical area stimulated (Stryker, 1982; Stryker and Strickland, 1984; Murayama et al., 1997).

Intracortical stimulation of motor cortex also has been reported to result in organizational changes. Large representational changes were generated rapidly and were progressive but also reversible with chronic microelectrode stimulation in motor cortex (Nudo et al., 1990). Ojemann (1994) found that acute electrical stimulation of motor cortex can result in a rapid functional change. With initial electrical stimulation of motor cortex a discrete area of cortex could be identified that would elicit motor activity. With continued stimulation this area of cortex expanded significantly.

Most relevant to the present study are studies of the auditory system in which intracochlear electrical stimulation was shown to have a significant effect on the cochleotopic organization in the auditory midbrain of neonatally deafened cats

(Snyder et al., 1990, 1991). Chronic electrical stimulation of a specific cochlear region expanded the central representation of that area. Further, the electrical stimulation altered the temporal response properties of IC neurons (Snyder et al., 1995). Chronic stimulation increased the maximum frequency to which neurons could follow in a synchronized manner. Vollmer et al. (In press) confirmed these results and further demonstrated that the temporal properties of the chronic stimulation applied determined the temporal response properties of IC neurons. Animals chronically stimulated with higher frequency signals had higher frequency following than animals stimulated with low frequency signals.

In addition to these temporal and spatial changes within the auditory system, morphological changes also have been observed in the electrically stimulated auditory system. Primarily, these studies have examined the consequences of electrical stimulation on spiral ganglion and cochlear nucleus neurons. Peripheral electrical stimulation of the auditory system has been reported to prevent degeneration of spiral ganglion cells in young and adult guinea pigs (Lousteau, 1987; Hartshorn et al., 1991) and in neonatally deafened kittens (Leake et al., 1991). Further, chronic stimulation lessens cell shrinkage in the cochlear nucleus (Chouard et al., 1983; Matsushima et al., 1991; Hultzcrantz et al., 1991; Lustig et al., 1994). Other studies however, have found no evidence of morphological changes within the peripheral auditory system of electrically stimulated animals (Shepherd et al., 1994; Araki et al., 1998; Li et al., 1997; In press). These conflicting results have led to some controversy as to whether or not stimulation by a cochlear implant can prevent SGC degeneration after deafness. Additional

studies clearly are required to better define the specific conditions necessary to induce putative neurotrophic effects of electrical stimulation in the maintenance of SGC.

1.4 Proposed study

The present study was designed to examine the consequences of chronic electrical stimulation in an *adult* animal model of deafness. Although the majority of previous studies have been conducted in neonatally deafened and young animals, it is also important to examine the effects of stimulation in the auditory system that has matured normally prior to deafness. Since cochlear implants are being recommended for more and more hearing impaired individuals, it is important to understand the consequences of intracochlear stimulation within both the central and peripheral auditory system.

This study was implemented in part to parallel the previously published data from our laboratory reported for an animal model of neonatal deafness. In order to examine the effects of stimulation on the mature deafened auditory system the adult cat was selected for study. Cats were bilaterally deafened, using ototoxic drugs, with the hypothesis that degeneration would be symmetrical between the two ears. Symmetrical deafness was a requisite not only in order to assess the impact of unilateral electrical stimulation on SGC survival but also because the auditory system has complex binaural pathways. Thus, unilateral input from a normal ear potentially could influence and/or maintain the functional organization and temporal response properties of the nervous system, thus ameliorating the effect of electrical stimulation.

Animals were unilaterally implanted soon after deafening, and chronic stimulation was initiated as soon as possible. It was expected that these animals would have relatively good ganglion cell survival after this short duration of

deafness and that stimulation could be initiated prior to significant SGC degeneration. The prolonged period of chronic stimulation was proposed to permit sufficient time for marked progressive SGC degeneration to occur in the control ear secondary to deafening. The hypothesis was that electrical stimulation would prevent degeneration in the implanted ear and that after a prolonged period, the neural degeneration in the control ear would show a significant difference.

The chronic stimulation signal was identical to one of the stimuli used in earlier studies of neonatally deafened animals in which increased survival of SGCs and alterations in the temporal and spatial response properties of IC neurons were observed. A quantifiable stimulation paradigm was selected to provide a known synchronized input signal (300 pps, sinusoidally amplitude modulated at 30 Hz). This signal was specifically selected to be temporally challenging to the central auditory system. That is, past studies have shown that 300 pps is close to the upper limit for neurons in the IC. Only a few IC neurons are capable of responding at or above this pulse rate of stimulation. Moreover, since cortical neurons respond best to AM signals at 8-12 Hz, the modulation envelope of this signal was considered to be temporally challenging to the central auditory neurons. Such temporally challenging signals also have been shown to be more effective in maintaining SGCs in the neonatally deafened and chronically stimulated animal (Leake et al., In Press).

Following chronic stimulation the spatial representation of electrical signals and neuronal response properties were examined in the inferior colliculus (IC). The IC was selected for study because it has a precise tonotopic organization

which can be used to infer relative frequency and selectivity of stimulation in deaf animals. In addition, the central nucleus of the IC receives information from many brain stem nuclei and is a mandatory relay to auditory cortex.

The EABR was used to determine threshold because it is a fast and accurate method for estimating behavioral threshold. Finally, the cochleae were examined and morphometric methods were used to evaluate spiral ganglion cell survival.

The following specific questions were proposed to be answered in this animal model.

1. *What are the response characteristics of IC neurons following many months of patterned chronic electrical stimulation?*

Snyder et al. (1995) examined the synchronized temporal firing of isolated single units in the IC and found that control (adult deafened) unstimulated animals had a maximum frequency following of 92 pulses/second (pps). However, chronically stimulated neonatally deafened animals had an average maximum frequency following (140 pps) that was significantly higher than normals. A more recent study by Vollmer et al. (In press) examined the temporal response properties of IC neurons in neonatally deafened animals that were chronically stimulated with different signals (low vs high frequency). In addition, they analyzed the responses based on neuron location within the IC. This study provided direct evidence that temporal response properties differ depending on the characteristics of the stimulating signal, with temporally challenging stimulation (e.g. 300pps/30 Hz) being more effective in altering response

properties. Chronically stimulated animals showed maximum frequency following that was significantly greater than control (unstimulated animals). In addition, their study examined the first spike latencies for the various groups of animals and found that neonatally deafened chronically stimulated animals had shorter latencies as compared to normal animals.

Again, these profound effects on the temporal response properties of IC neurons were observed following chronic intracochlear electrical stimulation of the neonatally deafened animal. The role of development on these changes has not been determined. Therefore it is important to examine and compare the effects of stimulation on the *mature* auditory system.

Given the extent of plasticity observed in other sensory systems in mature mammals and the profound changes observed in chronically stimulated neonatally deafened animals, it is hypothesized that similar stimulation will induce profound changes in the mature central auditory system.

2). Does chronic electrical stimulation alter the spatial representation of the cochlea within the inferior colliculus?

Snyder et al. (1990) also demonstrated an expansion of spatial representation of a chronically stimulated channel of a cochlear implant within the IC of neonatally deafened animals. Spatial tuning curves (thresholds vs depth in the IC) were significantly broader in animals that were chronically stimulated (normals = 0.64 mm; neonatally deafened= 1.51).

It is hypothesized that chronic stimulation of a specific channel of a cochlear implant in the mature animal also may result in profound functional changes in the central nervous system. Further, since intracochlear bipolar stimulation is expected to be fairly restricted it is hypothesized that expansion will occur only for the chronically stimulated electrode pair (1,2), and that the unstimulated pair (3,4) will not be effected.

3). If spatial selectivity and single neuron response characteristics are altered with chronic stimulation can these changes be recorded using the far-field recorded auditory brainstem response?

A fast, non-invasive method for assessing temporal resolution could be important for predicting benefits and for selecting rehabilitative methods in cochlear implant patients. In cochlear implant users it is known that temporal resolution is important for the perception of temporal pitch, prosody and speech, and that speech perception can vary widely in different individuals (Shannon, 1992). Moreover, modern cochlear implants have implemented processing strategies that utilize high pulse rate carriers (e.g. 800 pps) in an attempt to provide more detailed speech information to users. Some individuals have improved performance with such processing strategies, yet others do not obtain significant speech understanding even after years of use. It has been suggested that these differences in performance may be due to differing temporal resolution capabilities. Thus, easily evaluating temporal resolution may be a valuable tool

for adjusting processors and implementing rehabilitative methods in cochlear implant users.

One potential method for evaluating temporal synchronization is with the auditory brainstem response. Studies using acoustic stimuli have shown a decrease in the ABR waveform amplitude with increasing rate (frequency) of stimulation (Hall, 1992). It has been suggested that this decrease in amplitude is indicative of a degradation of temporal synchronization in the central auditory system (Laskey, 1996).

Therefore, the electrically evoked ABR to increasing rates of stimulation was examined as a method of determining temporal resolution. A specific goal of the present study was to evaluate changes in EABR response amplitude to pulse trains of increasing rates and to determine temporal synchronization before and after chronic electrical stimulation. In addition, it may be of interest to compare the far-field data with measures of the spatial selectivity and to single neuron response data.

4) Does chronic electrical stimulation have a protective effect on spiral ganglion cell survival?

Several studies have reported that electrical stimulation prevents the retrograde degeneration of SGCs in the stimulated ears of deafened animals (Lousteau, 1987; Leake et al., 1991; Hartshorn et al., 1991). Recently, Leake et al. (In press) reported a 21% increase in cell survival in the stimulated ears of neonatally deafened cats.

In contrast, another recent study in cats reported no increase in SGC density after chronic stimulation, although a significant increase in cell size was observed in the stimulated ears (Araki et al., 1998).

Thus, one goal of this study was to examine whether or not chronic electrical stimulation could prevent or ameliorate the SGC degeneration that is expected to occur as a consequence of profound deafness in adult cats.

2. 0 METHODS: Overview

Six healthy adult cats were purchased from commercial vendors and housed in the Animal Care Facility at the University of California San Francisco. All experimental procedures were approved by the University's Committee on Animal Research and met the NIH guidelines for the care and use of animals in research. To ensure that animals were fully mature, females were selected that weighed more than 2.5 kg and males weighed more than 3.0 kg. All animals had normal hearing sensitivity as evidenced by auditory evoked potentials. After ABR measurements were completed, the animals were deafened by ototoxic drug administration. After recovery from the deafening procedure, animals were implanted unilaterally with a specially designed cat electrode. Physiological thresholds for electrical stimulation were determined subsequently by measuring electrically evoked auditory brainstem responses (EABR). In addition to the primary experimental group of 6 cats, 5 other adult animals were selected, deafened and implanted using identical procedures. These 5 cats were not part of the extended chronic stimulation experiment, but they were studied in order to supplement data on the deafening procedure and the initial EABR data.

The six cats in the primary experimental group were chronically stimulated using intracochlear electrical pulses for 4 hours/day, 5 days/week for 13 – 32 weeks. Stimulation intensities were set to 2 dB above EABR threshold. Immediately after implantation and prior to a final acute physiological experiment, EABR wave III amplitudes were measured to varying rates of stimulation for pulses presented at 6 dB above threshold. The rate of pulse presentation was increased from 10/second to more than 300/second in order to estimate the adaptation rate of the EABR response in these

prior normal cats. In the final acute physiological experiment single neuron responses in the inferior colliculus (IC) were recorded to both sinusoidal and pulsatile stimuli for 4 of the 6 stimulated animals. From these data the estimates of temporal resolution (maximum frequency following and latency) of single neurons and the spatial selectivity of the chronically stimulated channel of the cochlear implant were determined for these electrical signals. At the end of the physiological experiment animals were euthanized by overdose of barbituate and a transcardiac perfusion with histological fixative was carried out. Finally, the density of surviving spiral ganglion cells was estimated bilaterally using light microscopy and morphometric analyses.

2.1 Deafening

The adult animals were sedated with an intramuscular injection (IM) of ketamine HCl (33 mg/kg) and acepromazine maleate (0.1 mg). The forearm was shaved and an intravenous (IV) catheter inserted. Sedation was maintained with additional injections of ketamine or sodium pentobarbital (25 mg/kg, IV) as required. Baseline acoustic auditory brainstem response (ABR) intensity series were recorded (see section 2.2), and acoustic thresholds to clicks were determined visually for both ears. After acoustic thresholds were determined, these normal animals were deafened using an administration of ototoxic antibiotics. Most animals were deafened by coadministration of kanamycin and ethacrynic acid as described by Xu et al. (1993). In this case, kanamycin (300 mg/kg) was dissolved in sterile saline solution and injected subcutaneously. After a delay of 20 minutes an automatic infusion pump (Razel, Model A-99) set to administer 1 mg/minute of ethacrynic acid dissolved in saline (1 mg/ml) was started. Drug infusion was

continued until no ABR responses were obtained to clicks at equipment intensity limits (110 dB peSPL). ABRs were monitored for 4 hours after this criterion was reached to ensure that hearing did not recover. One animal was initially deafened with a single subcutaneous dose of kanamycin (400 mg/kg; S.C.) and aminooxyacetic acid (25 mg/kg) as described by Leake et al. (1987). However, hearing thresholds recovered over the course of several hours of monitoring. This animal was subsequently deafened with kanamycin and ethacrynic acid as described above. All animals were given intravenous or subcutaneous fluids and body temperatures were maintained by a heating blanket during recovery from anesthesia. After a 2 week recovery period the ABRs were reassessed. Once deafness was re-confirmed, profound hearing loss was assumed to be irreversible and animals were implanted with a cochlear implant.

2.2 Signal Generation and Evoked Potential Recordings

Acoustic and electrical auditory brainstem responses were recorded differentially from silver wires inserted through the skin (vertex - active; ipsilateral mastoid - reference; and contralateral - ground) amplified (100,000x) and bandpass filtered (0.01-10.0 kHz), using a battery powered preamplifier (DAM-50) and a plug-in oscilloscope amplifier (3A9 Tektronix). *Acoustic* stimuli (200 μsec clicks, 20/sec) were delivered through a canister headphone (STAX, model SMR-1/MK-2) coupled to the ear by a hollow ear bar inserted into the external ear canal. *Electrical* stimuli consisted of charge balanced (capacitatively coupled) biphasic square wave pulses, 200 μsec/phase, 20/sec delivered to the cochlear implant via a specially designed optically isolated, voltage-to-current amplifier (Vureck et al., 1981). This amplifier was calibrated prior to every

recording session to deliver 100 μAmp output for an input into a 10 kΩ resistence for 1 Volt peak-to-peak input. All stimuli were generated by a TMS 3200 PC based workstation driven by custom software.

For all evoked potentials (acoustic and electric) responses were averaged for 500-1000 stimuli using a 16 bit A/D converter IBM PC operating at a sampling rate of 30 kHz. Threshold was defined as the lowest intensity level at which a repeatable response was just visible.

In implanted animals, electrically evoked auditory brainstem responses (EABRs) were recorded at 6 dB above threshold to continuous trains of pulses presented with varying interpulse intervals (20 – 333 pps). The amplitude of wave III was measured peak-to-peak for each interpulse interval and the data were plotted as frequency versus response amplitude functions.

2.3 Cochlear Implantation

After allowing 1-2 weeks for recovery from the deafening procedure, the left cochlea of each animal was implanted with a specially designed UCSF feline scala tympani electrode (**Fig. 1**). The electrode consisted of four platinum-iridium wires embedded in a silastic carrier. Each wire ended in a ball contact that was 250 μm in diameter. The electrode contacts were numbered 1 through 4 with the most apical contact designated as #1. Electrode leads were coiled in a percutaneous cable that terminated in a microconnector (See Snyder et al., 1990).

For cochlear implantation the animals were sedated with ketamine (33 mg/kg, IM) and acepromazine maleate (0.1 mg, IM). The head and forearm were shaved and an

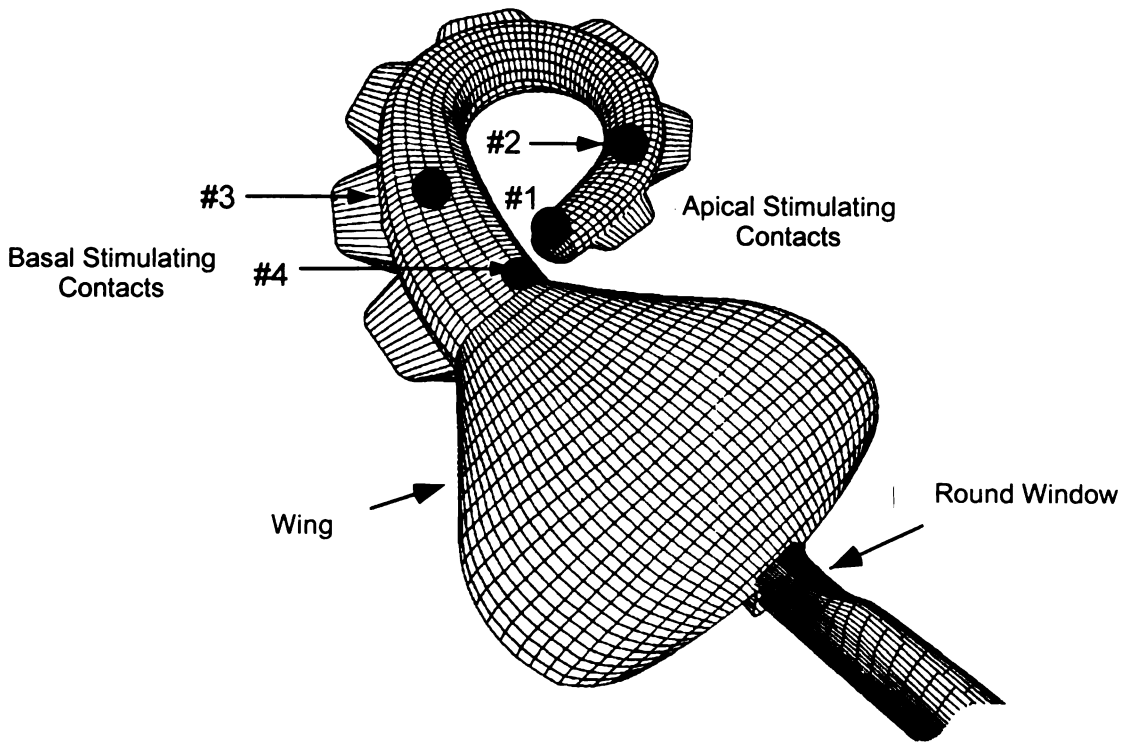


Figure 1. University of California San Francisco feline intracochlear electrode. In this study the apical electrode pair (1,2) was chronically stimulated. Each stimulating contact is 250 mm in diameter. The overall electrode length is 9 mm from the bottom of the wing to the apical tip. The center to center distance between electrode contacts within a pair (#1 to #2 and #3 to #4) is 1.0 mm. The distance between electrode contact #2 and #3 is 4.0 mm.

intravenous catheter was inserted into the forearm vein for fluid and drug administration. Intravenous sodium pentobarbitol was administered to induce and maintain a surgical level of anesthesia. The animal's head was stabilized in a mouthbar headholder and the scalp shaved and scrubbed for the sterile surgical procedure. Under asceptic conditions, a curved post-auricular incision was made through the skin. The erector pinnae and temporalis muscles and surrounding tissue were reflected rostrally from the midline and from the nunchal ridge. The strap muscles were reflected ventrally, and the auditory bulla was exposed and opened to access the round window. A small opening was made in the round window membrane and the intracochlear electrode gently inserted into the scala tympani. The intracochlear portion of the electrode had a small dacron fabric skirt which held it in place after Histocryl™ tissue adhesive was applied to fix it to the bone just ventral to the round window. A second cuff was secured to the periosteum of the parietal bone just dorsal to the bulla, and a third cuff was secured near the midline. The percutaneous connector was then routed through a second small incision in the skin at the midline of the neck caudal to the initial incision site. The temporalis muscle was re-approximated and sutured over the electrode cable. The incision site was lavaged with Nolvasan ™ (1%) or Bacitracin (25,000 units/ml) and closed in anatomical layers. Immediately following implantation physiological thresholds for electrical stimulation were determined by EABR measures. Buprenorphine HCl (0.005 mg/kg) for analgesia and prophylactic antibiotics were administered, and the animals were allowed to recover with continuous monitoring.

2.4 Chronic Electrical Stimulation

Electrical stimulation was initiated one week after cochlear implantation for six animals. These animals were stimulated in the laboratory for a period of 4 hours/day, five days/week. Detailed stimulation histories are presented in **Table 1**.

STIMULATION HISTORIES

Animal Number	Stimulation Period (days/wks)	Level (μAmp)	Duration of Deafness (weeks)
403*	66 /11	50-79	13
507	70/20	100-200	22
158	116/22	100	24
497	133/30	50-126	32
087	144/32	100-251	33
401	105/21	126-200	23

Table 1. Length of deafness and stimulation histories for the 6 adult cats included in this study.
** Initial attempt to deafen with kanamycin and aminooxycetic acid.*

Electrode impedances were recorded daily before and after stimulation. All animals received stimulation on the most apical electrode pair (1,2). The stimulus was a continuous train of sinusoidally amplitude modulated (SAM) pulses (200 μsec/phase) delivered at 300 pulses/second, 100% amplitude modulated at 30 Hz (**Fig 2**). The stimulus was computer generated using a digital-to-analogue converter running at 100k sample/second and its level adjusted using a digital attenuator. The attenuated signal was sent to a constant current stimulator calibrated to deliver 100 μAmp of current for 1volt peak-to-peak input. Stimulus intensity was adjusted so that the peak current was 2 dB above the animals' EABR threshold. The stimulator output was connected to the animal via the percutaneous connector. EABRs were recorded periodically (at intervals of about

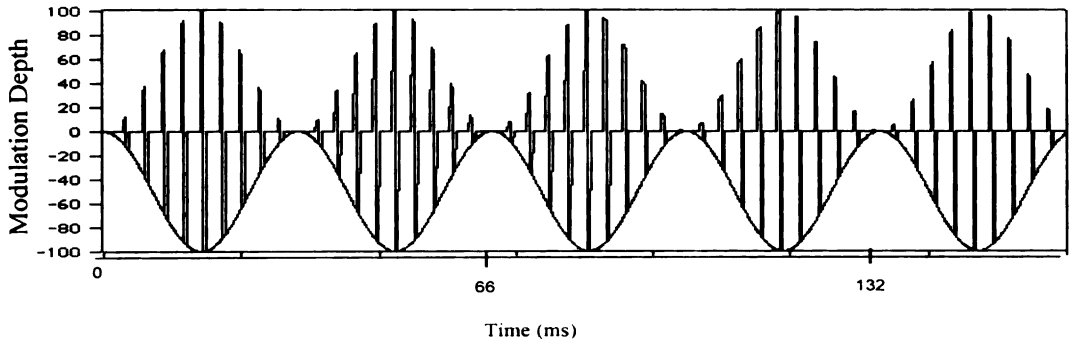


Figure 2. Waveform of the 300 pulses/second signal, sinusoidally amplitude modulated at 30 Hz. Chronic electrical stimulation was applied to the apical most electrode pair (1,2) at 2 dB above EABR threshold.

one month) and the current level of the stimulus was adjusted as necessary to maintain stimulation at 2 dB above the physiological threshold.

2.5 Acute Physiology

Final electrophysiological experiments to record single and multi-unit neuronal responses were conducted in 4 of the 6 stimulated animals. One animal (#507) damaged the cochlear implant and required reimplantation after approximately 12 weeks of stimulation. Due to extensive connective tissue within the scala tympani and difficulty in introducing a new implant, the round window was fractured. This resulted in cochlear pathology so severe that thresholds were markedly elevated and no acute physiological experiment could be conducted. The other animal (#403) died unexpectedly after only 11 weeks of chronic stimulation while it was sedated for electrode repair.

2.5.1 Inferior Colliculus Exposure

The four remaining animals were sedated with ketamine/acepromazine as previously described for implantation surgery. An IV catheter was placed in the forearm vein for administration of fluids and medications. To prevent brain edema, dexamethasone (1mg/kg; subcutaneously) and mannitol (250mg/ml, intravenous) were administered daily. Prophylactic antibiotics were given (cefazolin sodium; 100mg/ml, subcutaneously) twice daily through the duration of the experiment. Atropine sulfate (0.045 mg/kg, subcutaneously) was given as necessary to contol secretions. Sodium pentobarbital in lactated ringer's solution (1:20) was infused continuously (~4 ml/hour) via the intravenous catheter to maintain a surgical level of anesthesia throughout the experiment. The O_2 saturation, heart rate, respiration rate and temperature were

monitored continuously. The head and neck were shaved. A tracheostomy was performed and a tracheal tube inserted and sutured in place. The animal was stabilized in a standard mouth bar head holder. A lateral right sided craniotomy through the parietal bone just anterior to the tentorium was performed. The dura was opened and the underlying occipital cortex removed by aspiration to reveal the rostral portion of the right inferior colliculus and the right half of the tentorium. The bone of the inferior aspect of the tentorium was partially removed with a diamond burr and ronguered allowing direct visualization of the dorsolateral surface of the inferior colliculus.

2.5.2 Recording and signal generation

Electrical signals were generated and presented under computer control as described previously for EABR recording (see **section 2.2**). The output was converted from digital to analogue and the current signals relayed to an attenuator (Tucker-Davis, model PA-4). The output of the attenuator was forwarded to a stimulus isolation unit calibrated to deliver a 100 μAmp output for an input of 1.0 volt. The signal was then delivered to the intracochlear electrodes through a switch box connected by cable to the percutaneous connector passing through the skin at the nape of the animal's neck. The switch box facilitated delivering electrical stimuli to different pairs of intracochlear electrodes throughout the experiment. The signals were either 3 cycles of a 100 Hz sinusoid or 200 μs/phase biphasic square wave pulses.

IC responses were recorded using Paralene-coated tungsten micro-electrodes (0.9-2.0MΩ) inserted along a trajectory that was in the coronal plane and tilted off the saggital plane at an angle of 45 degrees from midline (**Fig. 3**). This allowed the

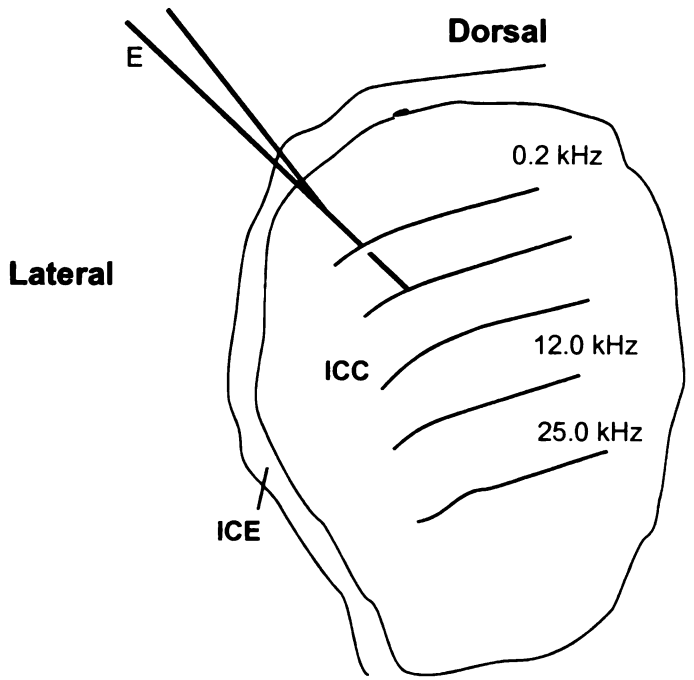


Figure 3. Schematic illustration of electrode penetration into a sagittal section of the inferior colliculus. Electrode trajectories were angled 45 degrees off of the sagittal plane and in the coronal plane. This angle of penetration is perpendicular to the topographic representation of ascending frequency (low frequencies more superficial or dorsolateral progressing to higher frequencies ventromedial) as shown by the contoured lines within the ICC (Webster et al., 1978; Brown et al., 1997). The reference electrode (not shown here) was placed in surrounding brain tissue.

E=electrode; ICC=Inferior colliculus central; ICE inferior colliculus external

recording of spike activity orthogonal to the cochleotopic frequency gradient (Snyder et al., 1990; Brown et al., 1997). The recording electrode was held in position by a micromanipulator and the depth was controlled remotely by a micropositioner (Kopf, Model 650). A second recording electrode, matched in impedance, was placed in the surrounding tissue to serve as a reference electrode for differential recording. The output of the electrodes was fed into a differential amplifier (DAM-50), amplified 1000x and bandpass filtered (0.1-10.0 kHz). Second stage amplification (100x) (Tektronix 5110) was utilized and the responses visualized on the oscilloscope. Action potentials (spikes) were isolated from the stimulus artifact and background noise with a window spike discriminator (BAK-DIS-1). Spike occurrence times were recorded with a resolution of 50 μs and post stimulus time histograms (PSTH) constructed. A silver wire electrode inserted through the skin at the neck served as a ground.

Single and multi-unit thresholds were determined at 100 μm intervals throughout the course of each penetration for combinations of stimulating electrode pairs. Response thresholds were determined using audiovisual criteria for 3 cycles of a 100 Hz electrical sinusoid (30 ms in duration) and a biphasic square-wave pulse (200 μsec/phase) with an interstimulus interval of 300-500 ms. In addition to these threshold measurements, single neuron responses were also recorded at every location where a unit could be isolated. After determining a unit's threshold for pulses (at 10-20 pps), the maximum frequency following capacity of each isolated neuron was determined by setting the stimulus intensity at 4-6 dB above threshold and recording responses to increasing pulse rates beginning at 10 pps and increasing the rate in steps of 20 pps until the unit no longer responded. Pulse trains were 320 ms duration with an intertrain interval of 1 second.

2.5.3 Analysis of temporal responses

Computer generated PSTH's were constructed using 20 stimulus repetitions and were used to calculate temporal following. The maximum frequency following (Fmax) of the neuron was the frequency to which the neuron responded in a synchronized manner. The custom software displayed the period histogram for each PSTH and determined the synchronization significance ($p<0.01$) using the Rayleigh test (Mardia, 1971). The highest frequency to which the neuron showed a statistically significant synchronized response was defined as Fmax (see **Figure 4**). In addition, fast Fourier transforms (FFT) were computed to verify estimates of temporal following.

For latency measurements, the first 60 ms of the data were plotted as period histograms for the responses to 20 pps stimuli (see **Figure 5**). Recordings with visible artifact and/or evoked potential were excluded from all data analysis. Latencies >4.5 ms were accepted as single unit responses, since responses less than 4.5 ms could be generated by synchronized evoked potentials from other (lower) auditory areas.

2.5.4 Analysis of threshold data (spatial tuning curves)

Several complete penetrations (5-8) were made through the IC of each cat. Threshold values obtained at 100 μm intervals were plotted as a function of electrode depth in the IC and spatial tuning curves were constructed for each IC penetration. These STCs were typically constructed for several electrode configurations including bipolar pairs (1,2; 3,4; 1,4) and/or monopolar stimulating electrode pairs for both pulses and sinusoids. Data were 3-point smoothed and the width of the curves for sinusoidal stimulation at 6 dB above minimum threshold were determined for each specific

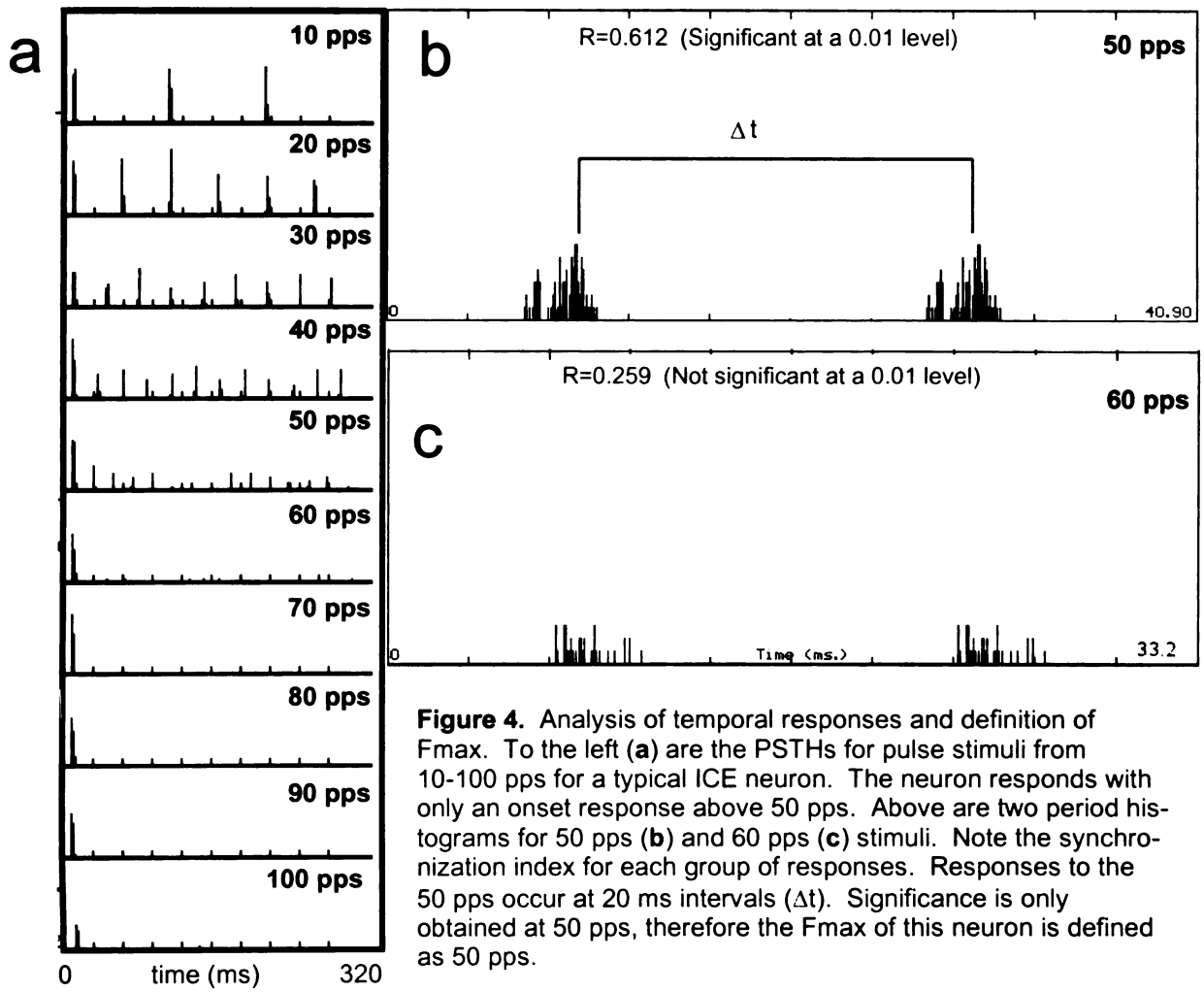


Figure 4. Analysis of temporal responses and definition of Fmax. To the left (**a**) are the PSTHs for pulse stimuli from 10-100 pps for a typical ICE neuron. The neuron responds with only an onset response above 50 pps. Above are two period histograms for 50 pps (**b**) and 60 pps (**c**) stimuli. Note the synchronization index for each group of responses. Responses to the 50 pps occur at 20 ms intervals (Δt). Significance is only obtained at 50 pps, therefore the Fmax of this neuron is defined as 50 pps.

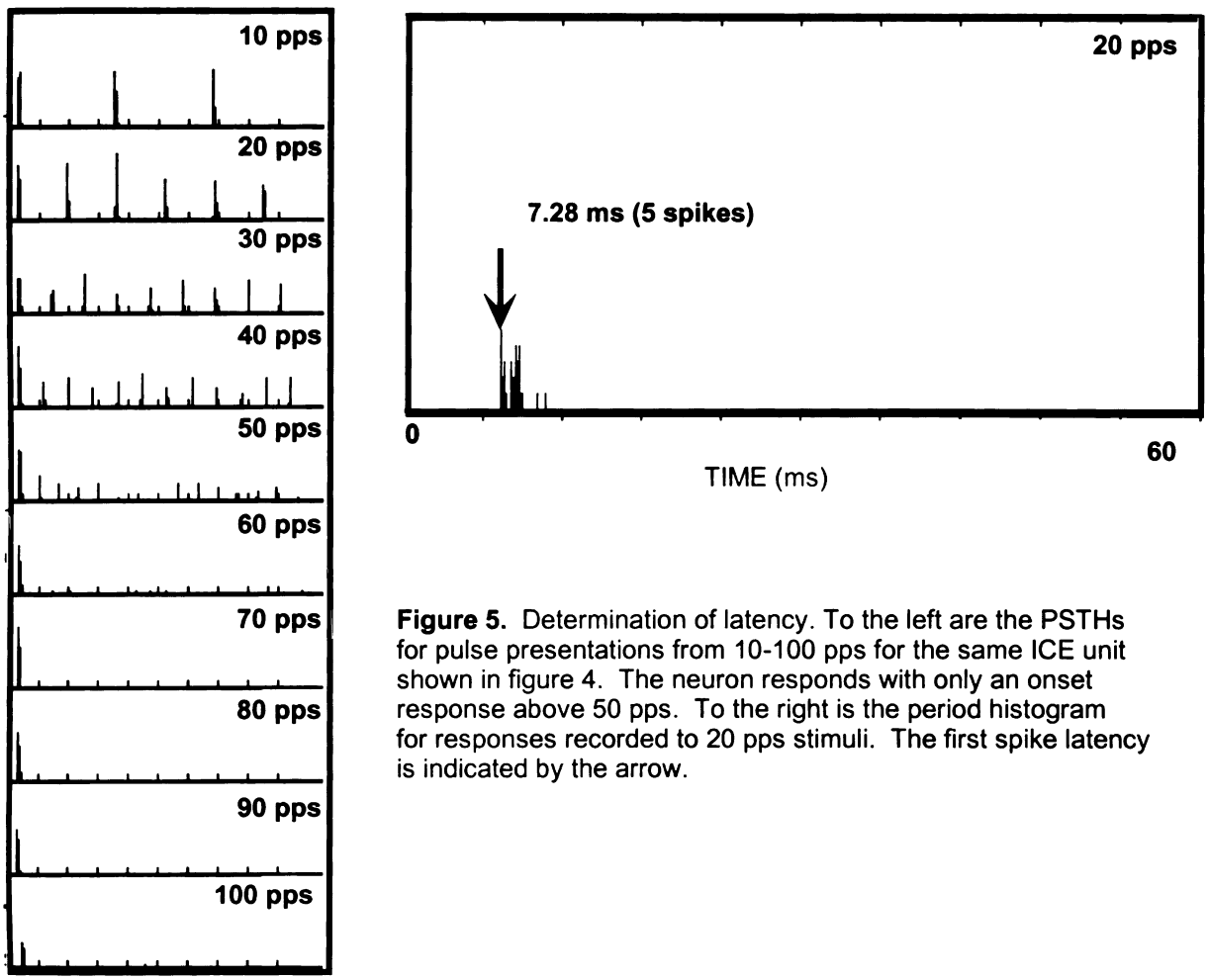


Figure 5. Determination of latency. To the left are the PSTHs for pulse presentations from 10-100 pps for the same ICE unit shown in figure 4. The neuron responds with only an onset response above 50 pps. To the right is the period histogram for responses recorded to 20 pps stimuli. The first spike latency is indicated by the arrow.

electrode configuration. (For more detail see section 3.3). Mean STC widths were calculated in order to compare the spatial selectivity of stimulation in these animals with similar data reported in previous studies of normal and neonatally deafened animals. In addition, since chronic stimuli were pulse trains, the STCs for electrical pulses also were examined. The pulse STC widths were examined at the current levels used for chronic stimulation and were used to estimate the relative efficacy and selectivity of activation of the central auditory system during chronic stimulation.

Finally, the spatial tuning curves were used to determine the border between the external and central inferior colliculus. Typically the tuning curves are 'w' shaped with two distinct regions of low threshold and a high threshold region between the two locations of minimum threshold. This high threshold region indicates the border between the two subnuclei of the inferior colliculus, the external (ICE) and the central (ICC). Past studies have suggested characteristic differences between neurons located within the central and external nucleus in their response s to electrical stimuli (Vollmer et al., 1997; 1998). Once the border was determined neurons were classified as belonging to either central or external nucleus and the frequency following capability of these populations was analyzed separately.

2.6 Morphology

2.6.1 Tissue preservation

Following a final acute electrophysiology experiment the cochleas were perfused with a mixed aldehyde fixative (2.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1M phosphate buffer, ph=7.4). In unimplanted ears perfusion was performed through

the oval and round windows. In the implanted cochlea a small hole was drilled in the bone overlying the cochlear apex and the perfusion was performed through this opening and the oval window. This perfusion method allowed the electrode to remain *in situ*. Transcardiac perfusion with Ringer's solution followed by fixative containing 2.5% paraformaldehyde, 1.5% glutaraldehyde and 4% sucrose in 0.1 M sodium phosphate buffer was then performed. The cochleae were removed and immersed in fixative overnight. After perfusion, the brain was removed and placed in 40% sucrose in 0.1 M phosphate buffer. The brainstem containing the cochlear nucleus was blocked and stored in buffer for later sectioning and analysis.

2.6.2 Cochlea

After fixation the temporal bones were rinsed in 0.1 M phosphate buffer. The otic capsule was thinned with a diamond dental burr until the stria vascularis was visible throughout the spiral. Several small openings into the scala vestibuli were created to facilitate embedding and uniform staining. The basal turn of cochlea was opened in animals with *in situ* electrodes in order to visualize electrode contacts. A small dental burr approximately the size of electrode contacts was used to mark the position of each contact in the bone adjacent to the stria vascularis. The electrode was then withdrawn from the scala tympani. Specimens were post-fixed in 1% osmium tetroxide with 1.5% potassium ferricyanide (12 hours), decalcified in EDTA (0.2 M, 48 hours), dehydrated in ethanol and embedded in epoxy resin (LX112™).

Next, each cochlea was bisected on the mid-modiolar plane and each half-coil was removed and mounted in epoxy resin on a glass slide. The markers indicating electrode contact location were preserved in the surface preparation to relate electrode

position to histology of the organ of Corti and morphometry of the spiral ganglion. The basilar membrane was then measured and 0.5 mm segments removed (samples taken at 2 mm intervals) and remounted for sectioning. Several groups of 1 to 2 μm thick sections were cut in the radial plane at 50 μm intervals from each block. Sections were stained with toluidine blue and coverslipped in immersion oil.

To quantify spiral ganglion cell survival a point counting method described by Weibel (1979) was employed. Rosenthal's canal was centered under a 10 X 10 counting grid at a magnification of 25x in the microscope ocular (10x) (**Figure 6**). The volume ratio of the spiral ganglion was then determined by counting the number of grid line intersections which fell over cell somata and dividing by the total number of intersections which fell within the total area of Rosenthal's canal in a given section. Five to 10 sections were analyzed in each 10% segment of basilar membrane distance. This method has been used previously to provide normative data for the cat spiral ganglion and quantitative evaluation of spiral ganglion cell degeneration in adult cats deafened by administration of neomycin (Leake and Hradek, 1988) and in studies of the effects of electrical stimulation in neonatally deafened cats (Leake et al., 1991; 1992 and 1995).

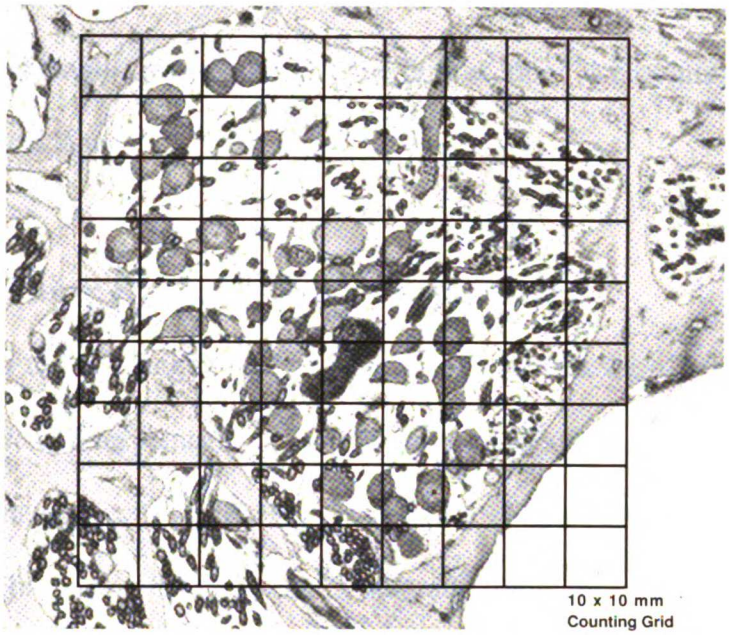


Figure 6. Point counting method for assessing SGC density. The 10 x 10 mm counting grid is positioned in the microscope ocular and is centered over Rosenthal's canal. The tissue visible beneath the grid is magnified 250x.

3.0 RESULTS

The results of this study are presented in 3 sections. Section 1 describes the results of the deafening procedure. Section 2 summarizes the physiological results including electrically evoked auditory brainstem response testing and acute physiological single unit recordings. The final section (3) presents the cochlear morphological data.

3. 1 Deafening: Kanamycin and Ethacrynic Acid

Prior to deafening procedures, binaural thresholds were determined in the adult animals by recording acoustic auditory brainstem responses to clicks presented at decreasing intensities (110 dB SPL to threshold). **Figure 7** shows an example of the ABR in a representative normal cat. At suprathreshold intensities the response consists of 4 positive peaks occurring within 5.0 ms. Waveform amplitudes decrease and response latencies increase with decreasing intensities. In this example, threshold to the acoustic stimulation was approximately 30 dB SPL. All animals included in this study demonstrated normal sensitivity with bilateral hearing thresholds $\leq$ 30 dB SPL (Evans, 1975).

Following threshold determination, kanamycin (300 mg/kg) was injected subcutaneously. Intravenous infusion of ethacrynic acid was started 20 minutes after the kanamycin was administered. Suprathreshold auditory brainstem responses were recorded at 2 minute timed intervals and monitored for an elevation in threshold as suggested by a decrease in ABR amplitude. The ethacrynic acid infusion was stopped once a change in threshold was noted. Typically, once the amplitude of the ABR

Auditory Brainstem Responses (ABR)

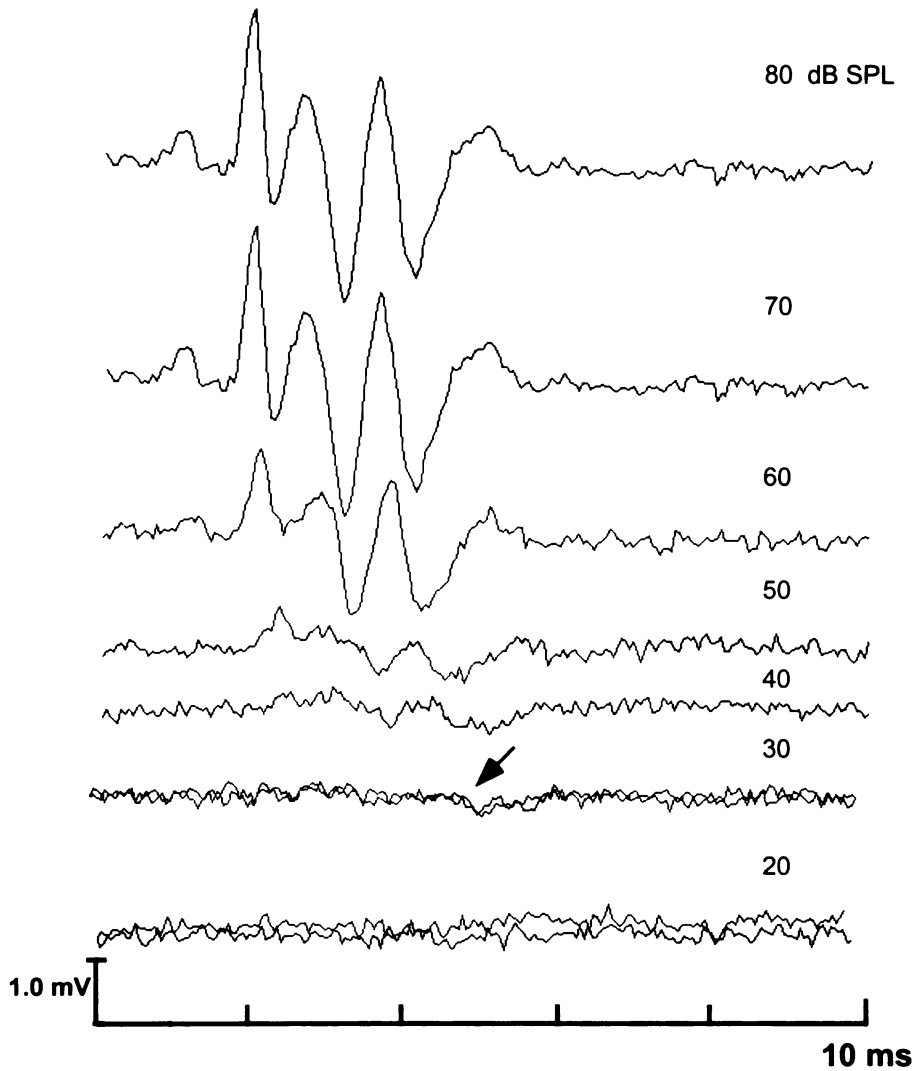


Figure 7. Auditory brainstem responses to clicks presented at varying intensities. Threshold (noted by arrow) in this example is approximately 30 dB.

showed an initial decrease, it continued to diminish at a very rapid rate. The absence of the ABR was monitored for approximately 4 hours or until the animal awoke from anesthesia. **Figure 8** illustrates an example of the effect of kanamycin injection and ethacrynic acid infusion on the click evoked ABR. An initial large amplitude ABR response can be observed for stimuli presented at –20 dB of attenuation (90 dB SPL). The response was maintained for 40 minutes. By 42 minutes (after initiation of ethacrynic acid infusion) there was an approximately 75% decrease in waveform amplitude and the ethacrynic acid was discontinued. No responses were present at 46 minutes after ethacrynic acid infusion was initiated, and re-testing at 240 minutes also failed to elicit a response. For 8 of 9 animals this protocol produced a consistent bilateral decrease and complete loss of auditory brainstem responses. In one animal the ABR recovered within 15 minutes after cessation of ethacrynic acid infusion. The infusion was restarted with a subsequent loss of the ABR after 12 minutes. No further recovery of hearing occurred.

Table 2 summarizes the pharmacological requirements that were necessary to induce and maintain deafness in 11 animals. The average time after beginning ethacrynic acid infusion until deafness was confirmed was 79 minutes (20 mg/kg) with a range of 50-104 minutes (12-27 mg/kg). Although the amount of ethacrynic acid needed to deafen individual animals varied considerably, there was a fairly strong correlation ($r = -0.60$) between body weight and the required amount of ethacrynic acid (see **Fig. 9**). The greater the weight of the animal the lower the dosage (per kg of body weight) of ethacrynic acid necessary.

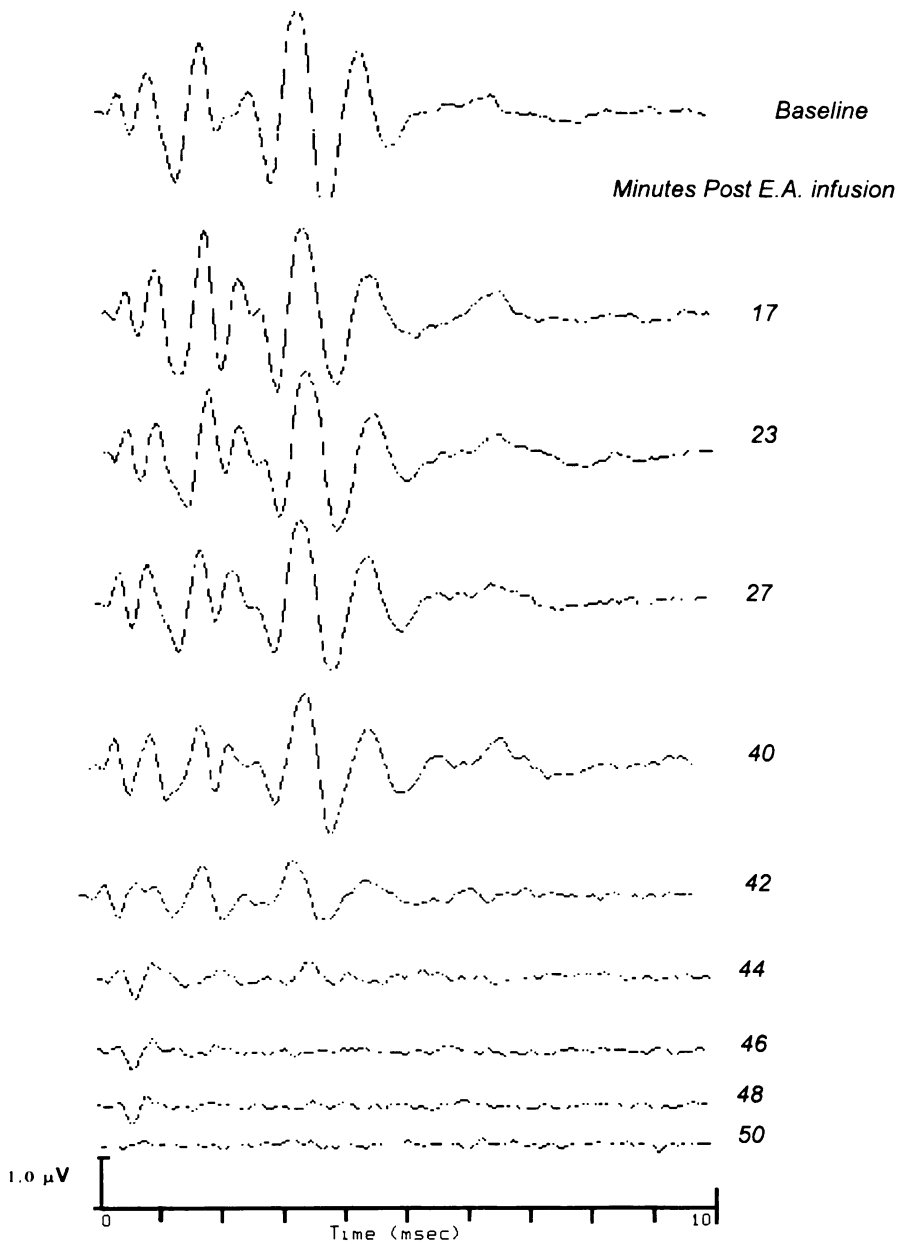


Figure 8. Example of auditory brainstem response (ABR) at baseline and following administration of kanamycin and ethacrynic acid. The intensity level for stimulation was 80 dB SPL.

Subjects	Weight (kg)	Kanamycin (mg)	Ethacrynic Acid (mg)	(mg/kg)	
403	5.0	1500	96	19.2	
507	3.6	1080	50	13.89	
158	4.9	1470	59	12.04	
497	2.7	810	68	25.19	
087	4.8	1440	100	20.83	
401	4.1	1230	104	25.37	
Additional Subjects					
116	3.4	1020	82	24.12	
114	2.9	870	78	26.90	**
115	4.7	1575	85	18.01	
122	4.2	1260	55	13.10	
645	5.1	1530	90	17.65	

**Restarted pump when some hearing recovered.

Table 2. Body weight of individual animals (kg) and actual amounts of kanamycin and ethacrynic acid necessary for inducing profound deafness.

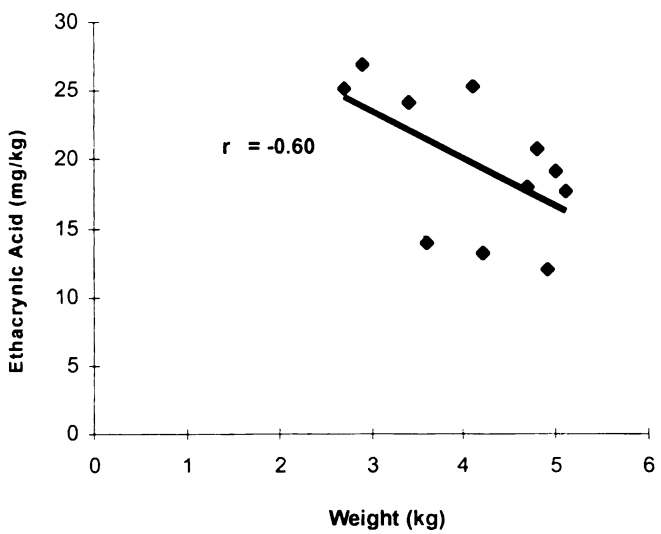


Figure 9. Amount of ethacrynic acid required to deafen individual animals as a function of body weight. Regression line shows a negative correlation between the dosage (mg/kg body weight) of drug required for deafening and the weight of the animal.

3.2 Physiology

3.2.1 Electrically Evoked Auditory Brainstem Response

Electrically evoked auditory brainstem responses (EABR) were recorded following implantation, at various time intervals during the chronic stimulation period, and just prior to the final physiological experiment. Initial threshold measures were used to set the intensity levels for chronic stimulation. Periodic measures were made to assure that electrodes were functioning properly and that the auditory system was being stimulated at the appropriate level (2 dB above threshold). Further, final EABR thresholds were used for comparison to single unit thresholds recorded within the IC of the same animal.

EABRs to biphasic pulsatile stimulation (0.2 ms/phase, 20 pps) of the apical 1,2 and the basal 3,4 electrode pairs were recorded. EABR waveform morphology and latencies were similar for all animals and for both stimulating electrode pairs (see **Fig. 10**). However, the absolute EABR threshold varied considerably between animals (50 – 634 μAmps) and between stimulating electrode pairs (12-505 μAmps) (see **Table 3**).

The average *initial* thresholds for pairs 1,2 and 3,4 were 84.35 and 203.91 μAmps, respectively, with greater variability noted for stimulating pair 3,4. In all but one animal stimulating electrode pair 1,2 had lower thresholds than pair 3,4 immediately following cochlear implantation. Over time all animals demonstrated some increase in threshold for the chronically stimulated (1,2) electrode pair, but the degree of elevation varied greatly among individuals. Thresholds for pair 3,4 elevated over time in 2 subjects but decreased in the others. EABR thresholds obtained at the

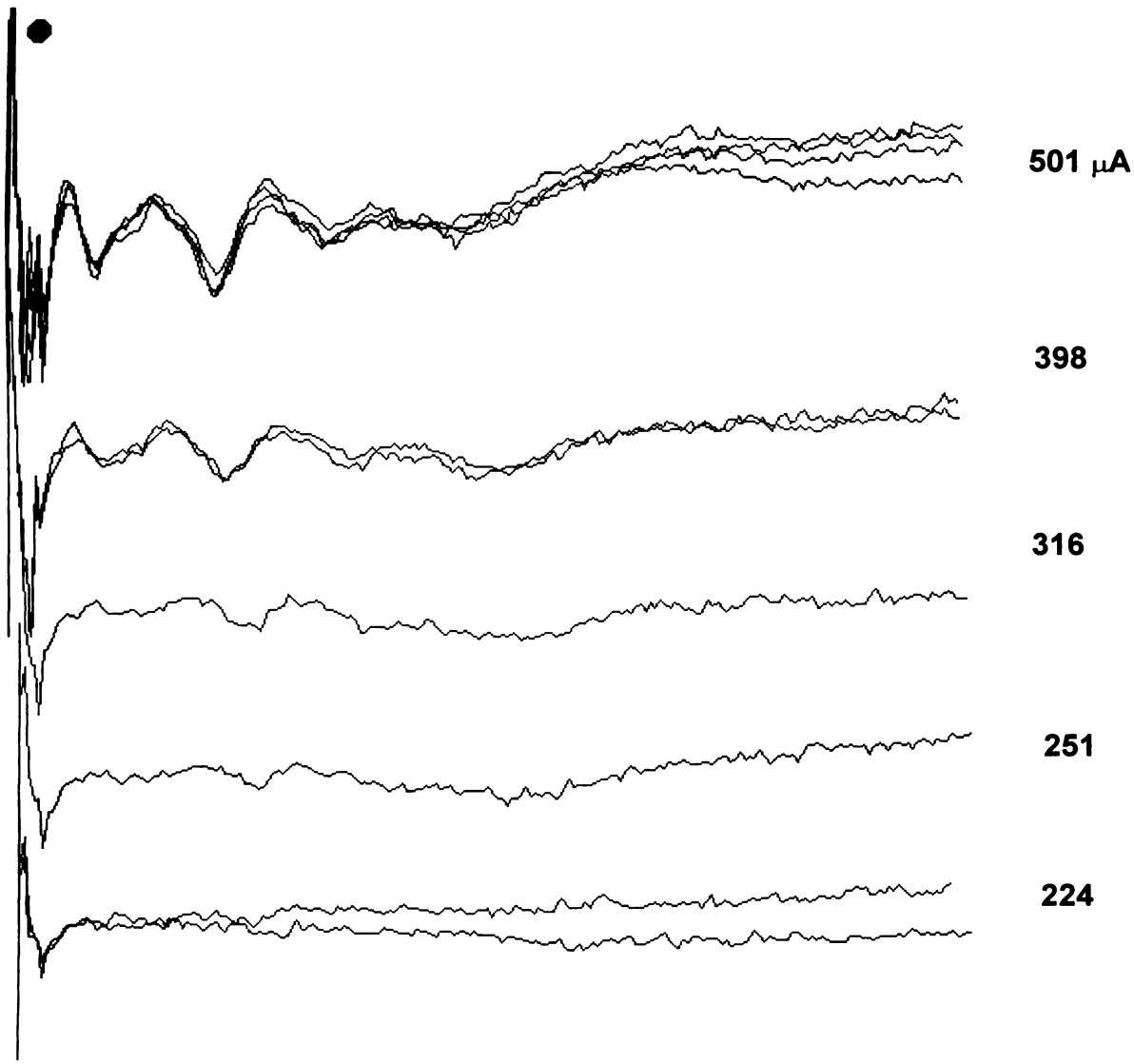


Figure 10. Electrically evoked auditory brainstem response (EABR) to varying levels of stimulation. The multiple recordings at 501 μAmps demonstrate the repeatability of the electrically evoked responses.

a.	Initial			b.	Final	
ANIMAL	1,2 (μA)	3,4 (μA)	Difference (μA)	1,2 (μA)	3,4 (μA)	Difference (μA)
403*	40	251	211	No final data		
507	79	251	172	158	*	
158	158	251	93	251	158	-93
497	50	100	50	100	89	-11
087	79	158	79	251	398	147
401	126	631	505	158	200	42
159*	126	251	125	No final data		
114	32	50	18	Currently in study		
115	63	100	47	Currently in study		
122	63	100	47	Acute		
645*	112	100	-12	Acute		
Average	84	204	124	184	211	73

Table 3. EABR initial (a) and final (b) thresholds in microAmps (μA). Initial threshold data were obtained immediately after cochlear implantation and are compared to the values obtained prior to final acute physiology experiment. Data are presented separately for stimulating electrode pairs 1,2 and 3,4, and for the difference between them (threshold for pair 3,4 less that for pair 1,2).

final testing session ranged from 89-398 μAmps. A comparison of mean initial and final EABR thresholds for pair 1,2 reveals an increase in threshold for pair 1,2 on the average of 87 μAmp.

3.2.2 Effects of auditory brainstem response amplitude to increased rates of intracochlear electrical stimulation

Currently there is no efficient method for assessing temporal resolution in cochlear implant users. Since it has been suggested that ABR amplitude decrease with increasing stimulation frequency may directly reflect temporal resolving capability, it was hypothesized that individual differences in amplitude for varied electrical

stimulation frequencies also may reflect or predict an individual's temporal resolution capability. Such a non-invasive method of assessing temporal resolution could be a valuable tool not only in predicting performance, but in the implementation of speech processing strategies and/or rehabilitative approaches.

To evaluate the EABR as a noninvasive method for assessing temporal resolution, EABRs were recorded at 6 dB above threshold to increasing rates of pulsatile stimulation (20-250 pps). These interpulse interval series were recorded immediately after cochlear implantation and just prior to the final physiological experiment. An example of EABR waveforms to varying rates of stimulation is presented in **figure 11**. Despite the marked decrease in amplitude for increased rates of stimulation, the response latencies changed only slightly. This finding contrasts with what is observed with acoustic stimulation, which typically shows a decrease in response amplitude accompanied by an increase in latency.

In order to compare EABR responses between animals and to document possible longitudinal changes within individual animals, the robust, highly repeatable wave III was selected for quantitative analysis at varying rates of stimulation. EABR wave III amplitudes were measured peak to peak, normalized (highest amplitude = 1.0) and plotted as a function of stimulation rate. **Figure 12ab** presents the normalized wave III amplitudes for nine animals recorded immediately after cochlear implantation. (**12a** data were recorded from Group 1 animals, for which final data following chronic stimulation were also available.) In all animals the amplitude progressively decreased with increasing stimulation rate. At approximately 250 pps only minimal or no response could be recorded. Although there is considerable jitter

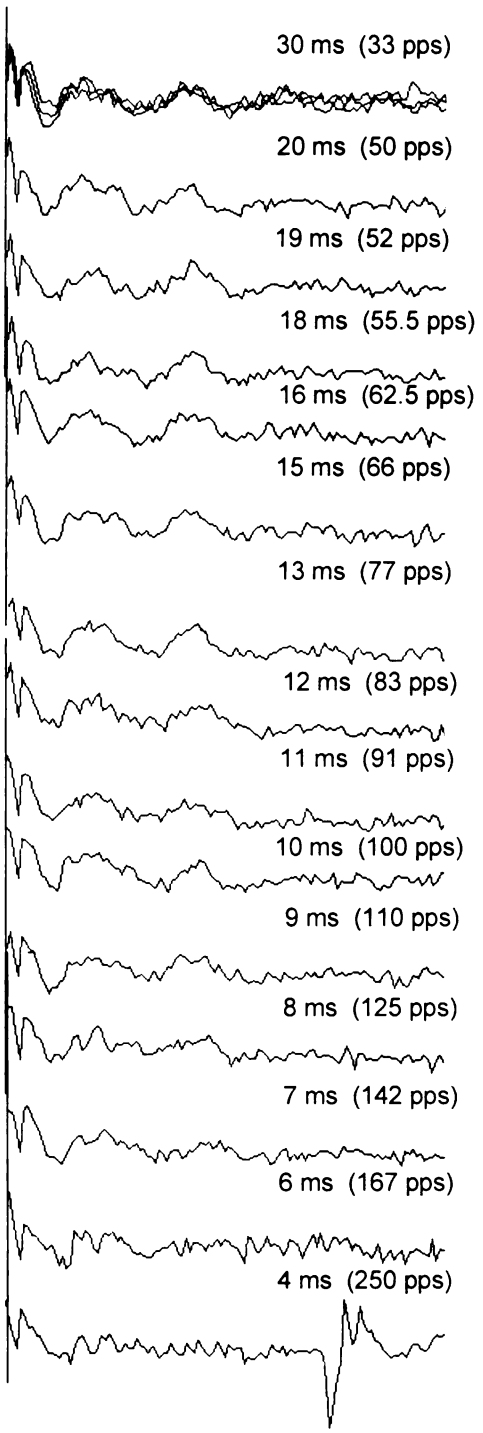


Figure 11. EABR responses to increasing frequency of stimulation. Responses are labelled with the interpulse interval (ms) and rate (pps).

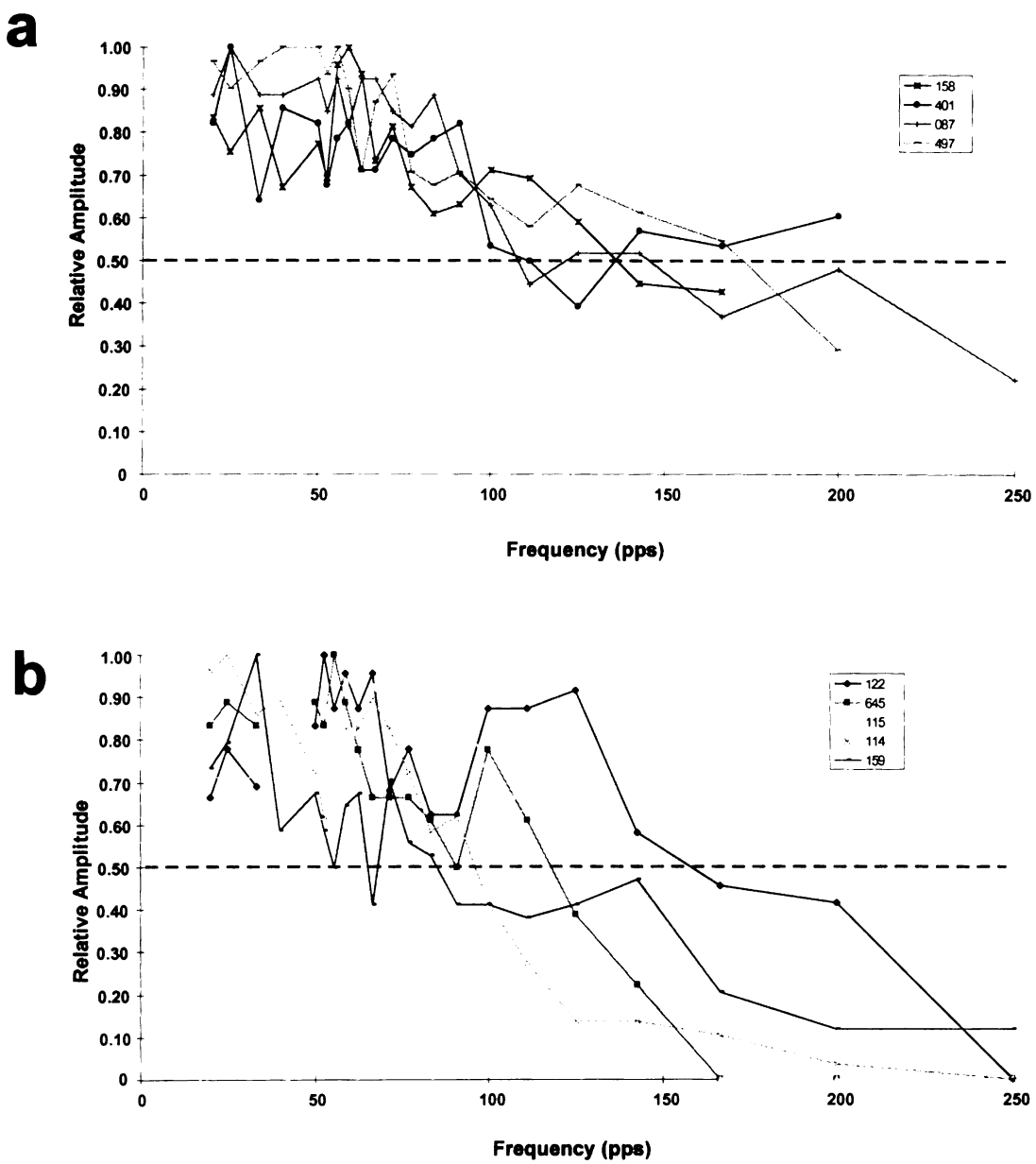


Figure 12ab. Relative EABR amplitudes (wave III) plotted as a function of rate of stimulation. Animals have been separated here into two groups to facilitate comparison of individual data. Group **a** are animals for which final data also are available. A dashed line indicates half relative amplitude.

in the data, the results show large and fairly consistent wave III amplitudes up to approximately 100 pps stimulation, followed by a significant and rapid decrease in response amplitude to the increasing stimulation rates. Animal #114 had a dramatic decrease in response amplitude at approximately 75 pps, with barely identifiable responses above 200 pps. At 250 pps no responses could be recorded. Similarly subject # 645 had responses to approximately 100 pps with a dramatic decrease thereafter. Animal #401 showed a gradual progressive decrease in amplitude all the way down to 200 pps.

Figure 13 presents the normalized EABR wave III amplitudes for the varying rates of stimulation recorded from animals (**group 1**) following several months of chronic stimulation. Three of four animals showed similar amplitude vs. frequency curves, displaying a drop in amplitude at approximately 90 pps then a gradual decrease to 250 pps. One animal, #93-087, showed much less of a systematic decrease in amplitude up to the highest measurable rate. The mean amplitudes for the 4 animals at the varying rates are shown by the heavy black line and show that the amplitude decreases gradually to about 40% at 250 pps.

To standardize the comparison between animals and within animals and to allow comparison with other measurements of temporal resolution (e.g. temporal data recorded from IC neurons during the final physiological experiment), the stimulation rate (frequency) at which the response amplitude was half of the maximum was determined. **Table 4** gives the individual half amplitude values for animals evaluated before and after chronic stimulation (n=4). Three of the four animals with before and after measures demonstrated an increase in maximum cut-off frequency following

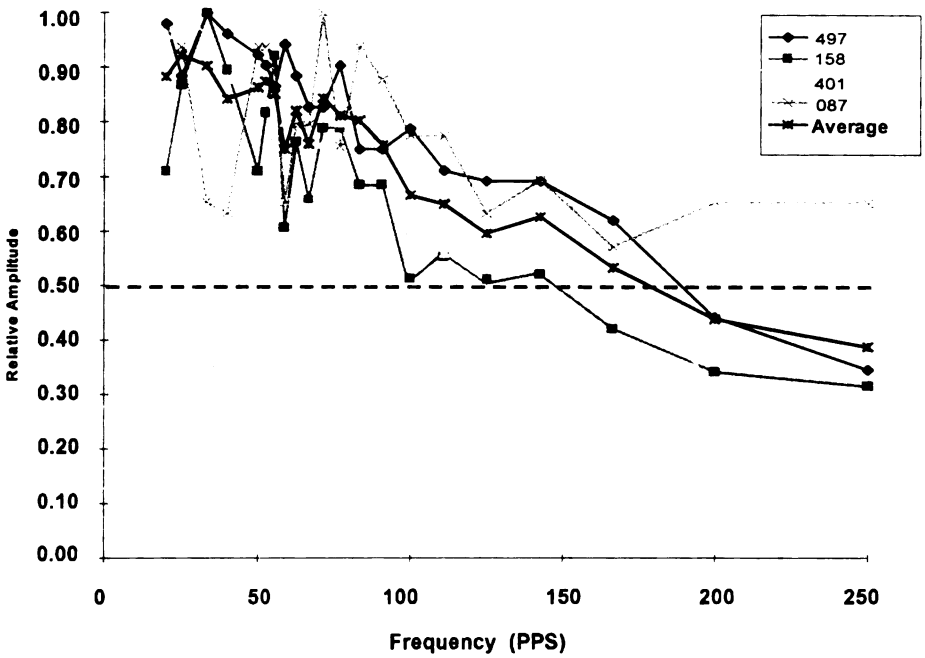


Figure 13. Final EABR wave III amplitudes recorded following several months of chronic electrical stimulation. Amplitudes are plotted as a function of varying rates of stimulation on electrode pair 1,2.

Animal Number	Half Amplitude (pps)	
	Initial	Final
158	140	147
497	170	187
087	145	>250
401	110	170

Table 4. The individual half amplitude values for animals evaluated pre (initial) and post (final) chronic electrical stimulation. Data are given in pulses per second (pps).

chronic stimulation. Animal #087 shows the greatest increasing in frequency following after 33 weeks of chronic stimulation. Animal #158 shows essentially no change in following rate after 24 weeks of chronic stimulation.

Figure 14 shows the initial and final amplitudes as a function of stimulation rate averaged for all available subjects. Response amplitudes remain relatively unchanged for both measurements up to approximately 75 pps. Above this frequency a pronounced decrease in amplitude was evident. The half amplitude cut-off points for initial (a) and final (b) average amplitudes are noted with black arrows. The average frequency at which the waveform becomes half of its maximum is 132 pps prior to chronic electrical stimulation and 174 pps following several months of stimulation. Although the sample size was too small for statistical analysis, the trend is consistent with an increase in the frequency following after chronic electrical stimulation.

3.2.3 Post-Stimulus Time Histograms (PSTH): Neuronal responses in the inferior colliculus to intracochlear electrical stimulation

Responses to intracochlear electrical stimulation were recorded in the contralateral inferior colliculus. A total of 191 neurons were studied in the 4 animals. For these single unit studies stimuli consisted of square-wave charge balanced, constant current, biphasic pulses (200 μsec/phase) and were presented at 6 dB above response threshold. To examine temporal resolution, maximum frequency following (Fmax) to pulse trains of increasing frequencies (steps of 10 pps) and onset latencies to pulse trains presented at 20 pps were determined for every recorded unit.

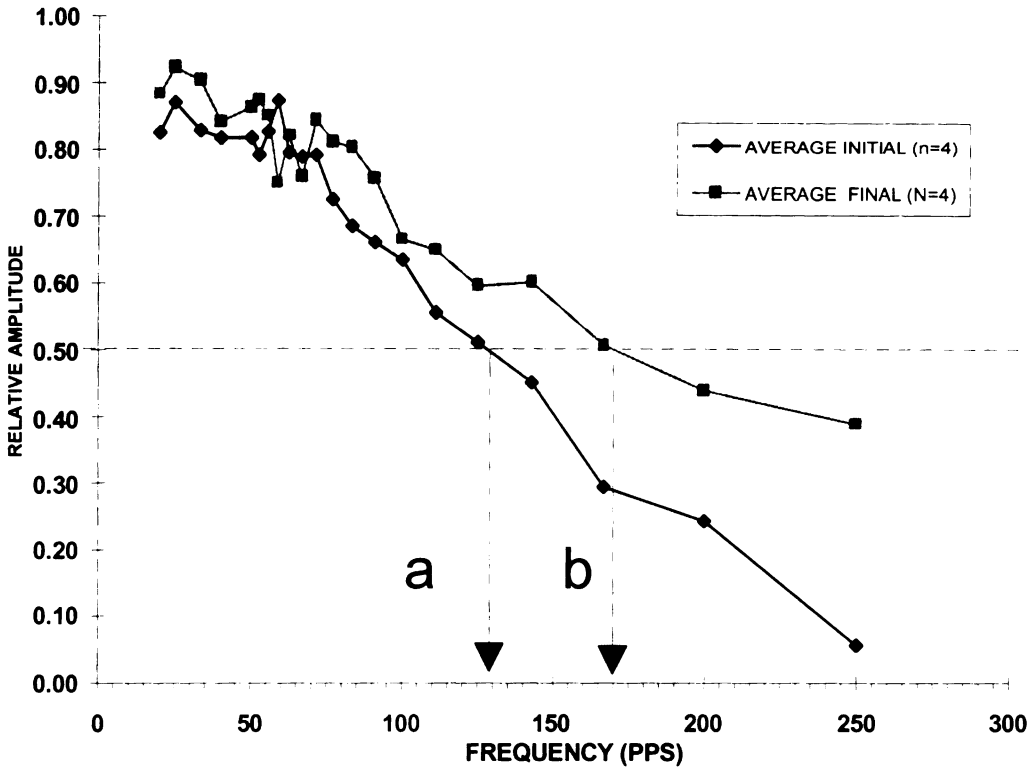


Figure 14. Mean relative amplitudes of EABR wave III plotted as a function of rate of stimulation. Curve **a** is data obtained prior to chronic electrical stimulation and curve **b** is data recorded following several months of stimulation.

Since recent studies have suggested that there are characteristic differences in responses recorded from the external nucleus (ICE) as compared to response in the central nucleus (ICC) of the inferior colliculus (Vollmer et al., 1998), unit data were analyzed separately for the two nuclei. Briefly, spatial tuning curves were generated by determining the response threshold for sinusoids (100 Hz) and pulses (0.2 ms/phase) at 100 micron intervals (Snyder et al., 1990). These tuning curves typically had 2 distinct minimum threshold locations reflecting the distinct tonotopic organization of each nucleus. The relatively non-responsive area between the two minima was defined as the border between the ICE and ICC (Vollmer et al., In press). (For more detail see section 3.3).

3.2.3.1 Maximum Frequency Following (Fmax)

The maximum frequency following was determined by examining neural responses to increasing pulse frequencies. The frequency at which a neuron responded in a synchronized manner ($p<0.01$) was obtained from the period histogram (see **Figure 4; section 2.5.3**). In addition, the phase locking of the neuron was confirmed by computer-analyzed fast Fourier transform. Neuronal responses which were only present to the first pulse of the stimulus train were not included.

Figure 15a and **b** are examples taken from one animal, showing PSTHs recorded from two different neurons within the central nucleus. The neuron in **figure 15a** is an example of a unit with relatively low temporal resolution. This unit has an Fmax of approximately 70 pps. Responses recorded above that stimulation frequency

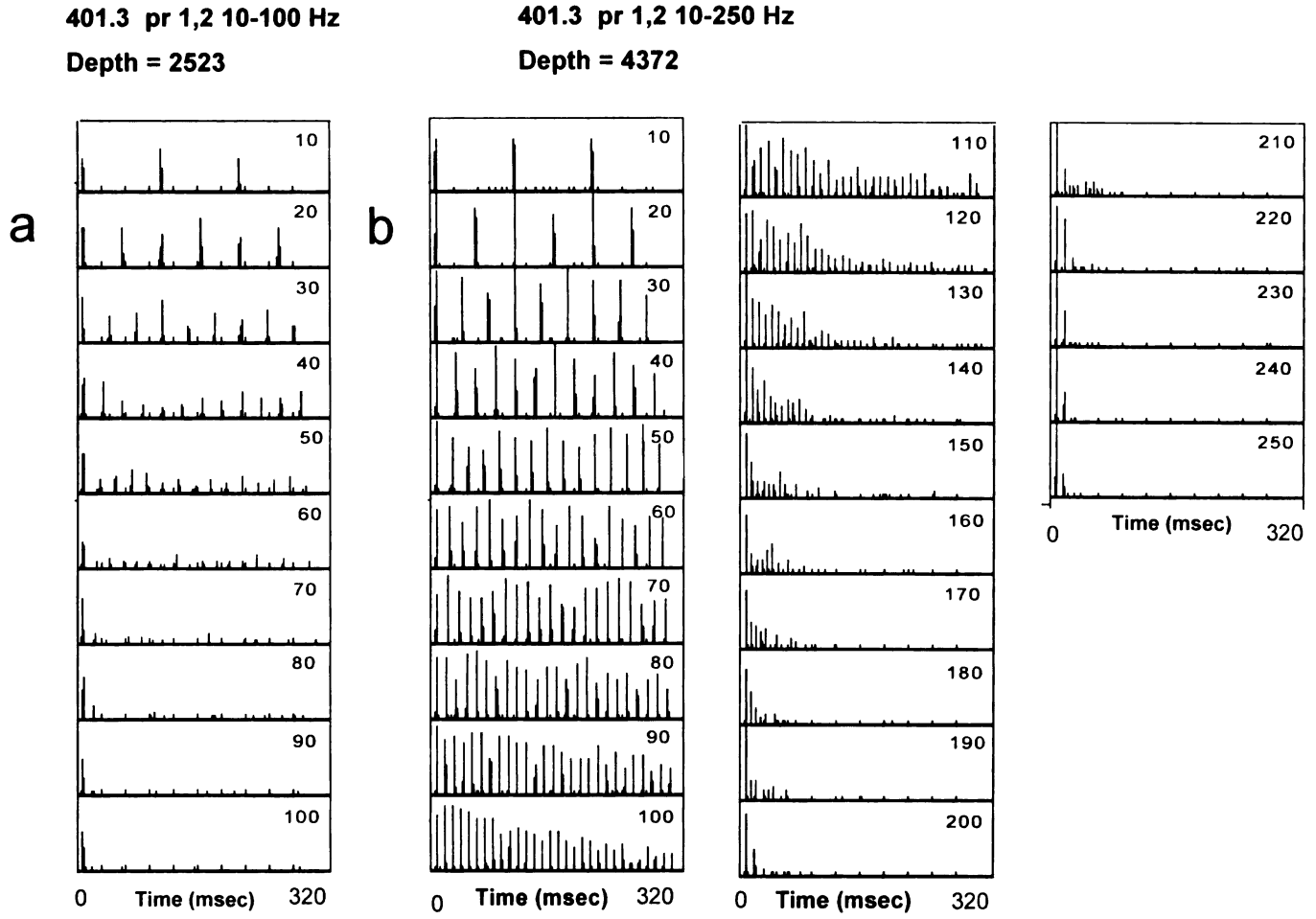


Figure 15ab. Responses of two neurons located in the central IC. Responses are recorded from electrode pair 1,2 to increasing frequency of pulsatile stimuli. Unit to the left responds up to approximately 70 pps and the unit on the right to 190 pps.

fail to phase lock to all but the stimulus onset. The neuron in **figure15b** is an example of a high resolution unit. This unit has an Fmax of 220 pps.

The averaged Fmax for individual animals are presented separately for the ICE, ICC and for the entire IC in **Table 5** and **figure 16**. In every animal the units in the ICE had a lower maximum frequency following than units located in the ICC. In the pooled data this quantitative analysis of 44 units located in the ICE revealed an average Fmax of 54 pps. In contrast, for 148 units located in the ICC the average Fmax was 137 pps. This difference between the two subpopulations of IC neurons was highly significant ($p<0.001$). Since some previously reported data do not distinguish between units in the ICC and those in the ICE, the overall average Fmax for all IC units in individual animals and the group means are also presented **(Figure 16; Table 5)**.

	Animal #				
	158	**497**	**087**	**401**	**Average**
External Nuc. (n=43)	65	57	57.5	35	54 pps
Central Nuc. (n=148)	168	114	143	123	137 pps
Total Units (n=191)	137	91	132	108	117 pps

Table 5. Average maximum frequency following (Fmax) for neurons recorded from 4 animals. Data are presented in pulses per second.

Figure 17 presents the distribution of neurons responding at each specific Fmax value. It is clear that neurons in the external nucleus (darker shadings) respond

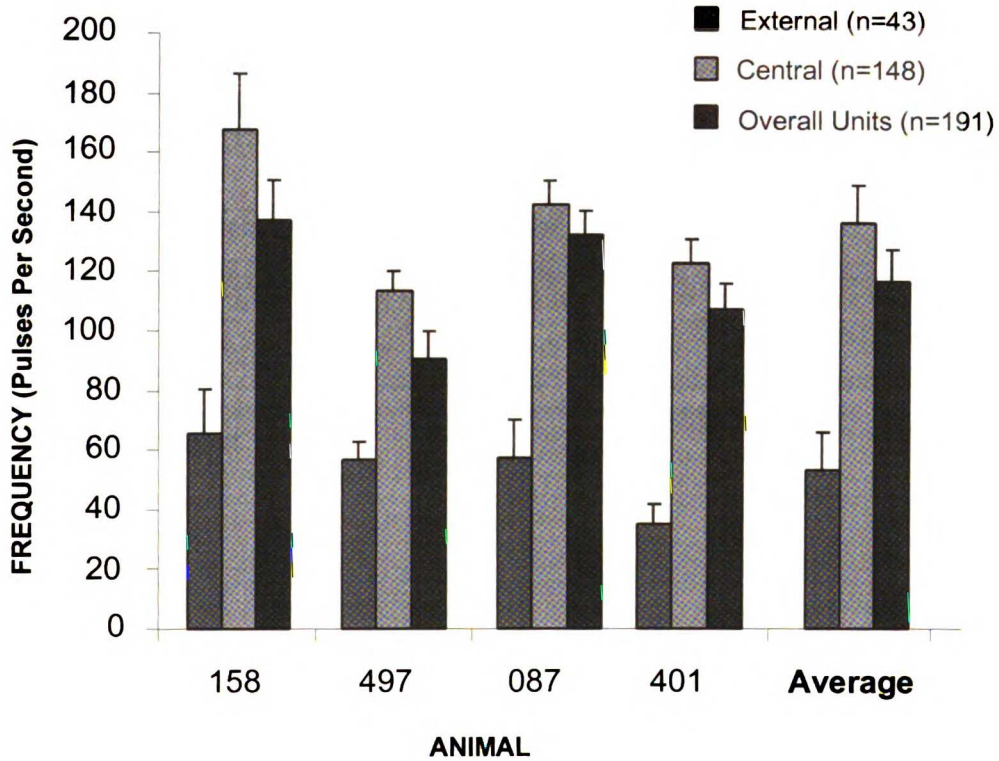
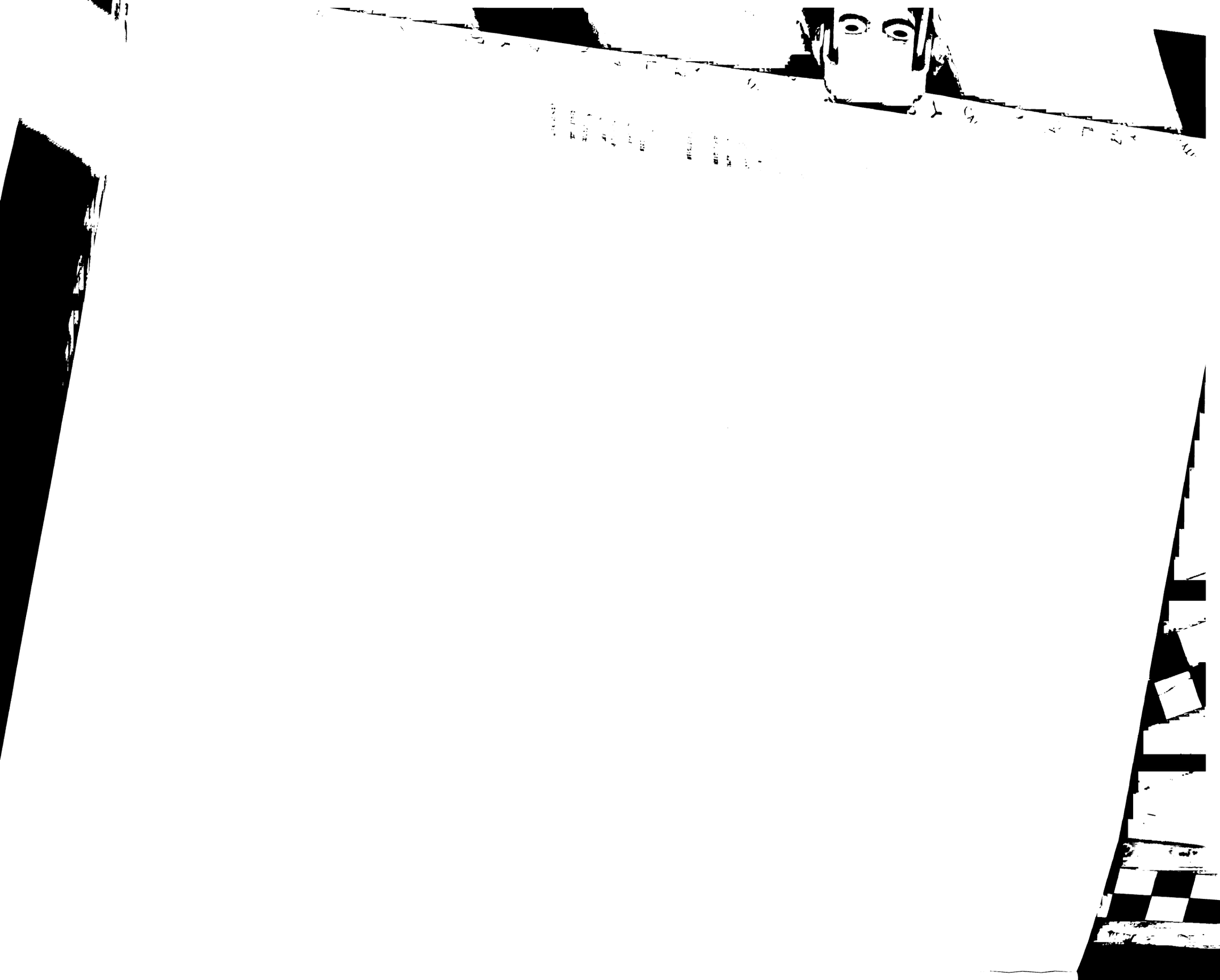


Figure 16. Average maximum frequency following (Fmax) for all units in the IC and the Fmax for units located in the external and central subdivisions.



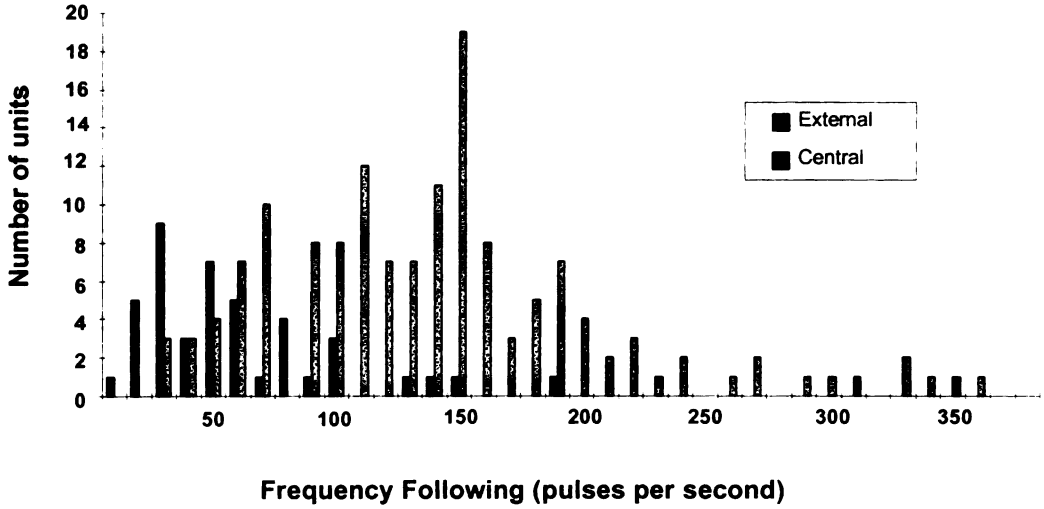


Figure 17. Distribution of Fmax for all units (n=191) separated by subdivision in the IC. Dark bars represent units recorded within the external nucleus and light bars are units from the central nucleus.

to a lower range of frequencies (10-180pps) than units located in the central nucleus (20-360 pps), where some units responded well above 200 pps. The median Fmax was 30 pps in the ICE and 150 pps in the ICC. As for the difference between mean values, the difference between median Fmax in the ICE and that in ICC was also highly significant ($p<0.001$).

3.2.3.2 Latencies

Onset spike latencies also were determined for all single neurons for responses to pulses presented at a rate of 20 pps. Onset latency data were available for 43 neurons within the external nucleus and 149 neurons in the central nucleus. **Figure 18** displays examples of period histograms for two units responding to 20 pps. Neuron (a) was a representative unit recorded from the ICE and had a response latency of 9.98 ms. Neuron (b) was recorded within the ICC had a response latency of 4.81 ms.

Figure 19 shows the average response latencies for units located in the external and central nucleus of the individual animals. In all animals units located in the external nucleus had significantly longer response latencies than units located in the central nucleus.

The distribution of units responding as a function of response latency is presented in **figure 20**. The graph shows that the first spike response latency for units located within the external nucleus ranged from 4.69 to 21.42 ms and the median latency was 7.2 ms (mean=8.78 ms). Units located in the central nucleus had first spike latencies which were distributed over a much narrower range of 4.52 to 8.91 ms. The median latency for central nucleus units was 5.69 ms (mean 5.85 ms). The

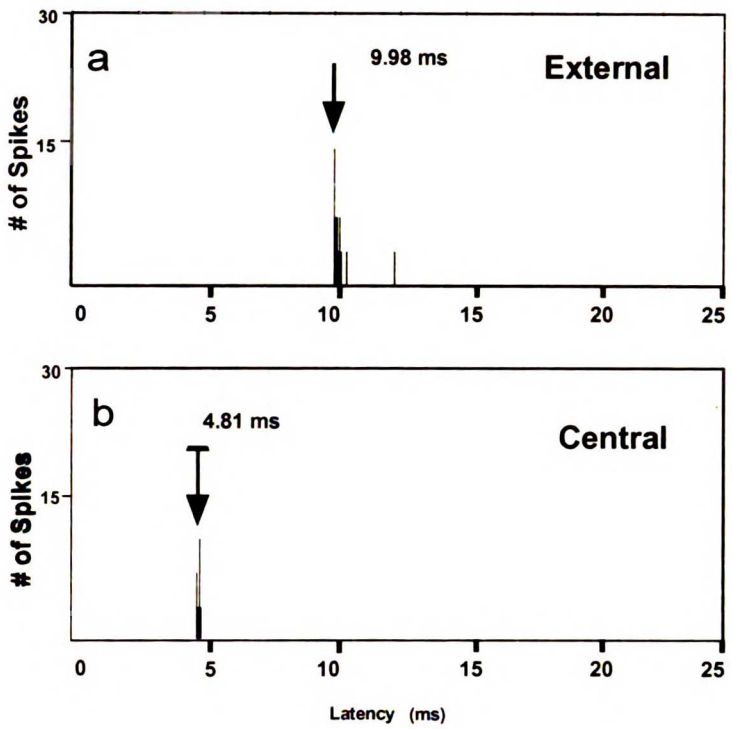


Figure 18. Examples of period histograms for two IC neurons. Neuron (a) was located in the external nucleus, neuron (b) was located in the central nucleus.

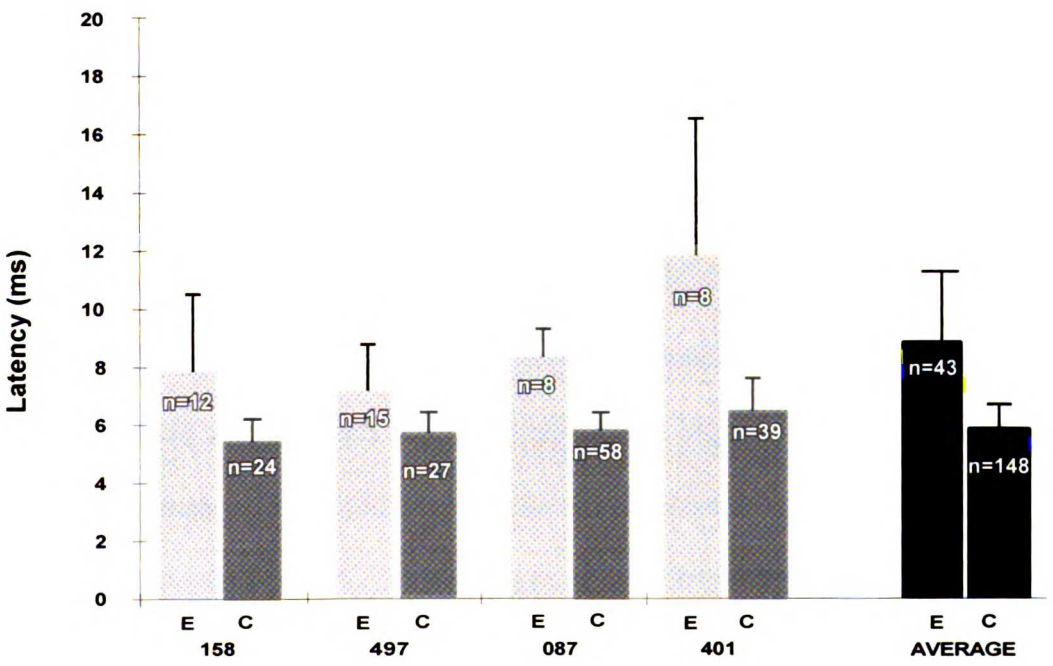


Figure 19. Average latencies and standard deviations for units separated by location (E=external; C=central) in the IC for individual animals.

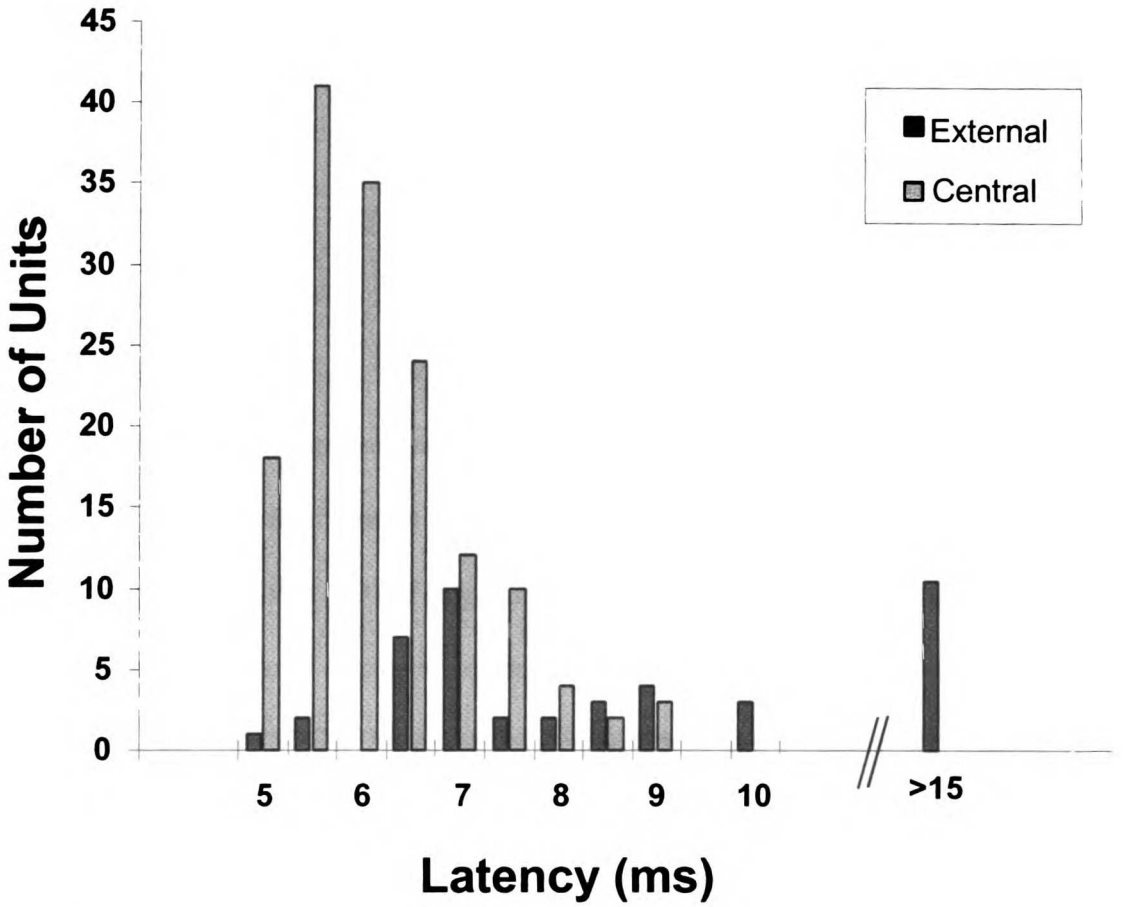


Figure 20. Distribution of latencies of neurons (n=191) in the external and central nucleus of the inferior colliculus.

difference in median latency between the external and central nucleus was significant (Mann-Whitney Rank Sum Test $p<0.001$).

3.2.4 Spatial Selectivity of Electrical Stimulation

Merzenich et al. (1973) first described the use of the spatial distribution of response thresholds in the IC to infer relative frequency and selectivity of electrical stimulation of the cochlea in deafened animals. Snyder et al. (1990) further developed this method to systematically quantify the relative selectivity of electrical stimulation. They plotted the thresholds (dB) of single and multi-unit clusters recorded every 100 microns for each of several electrode penetrations through the IC and termed these 'spatial tuning curves' (STC). These curves demonstrated that electrical stimulation delivered by one bipolar intracochlear channel at a specific location in the cochlea excited a relatively restricted area within the IC. In contrast, stimulation across a broad area of the cochlea using widely separated bipolar electrodes resulted in excitation that spread across a wider area of the IC. Measurement of the STC width at 6 dB above the best location provided a means of quantitative comparison of selectivity across and within animals.

Two exemplary spatial tuning curves (STC) generated in one penetration from an adult deafened animal in this study are displayed in **Figure 21**. In this example, response thresholds are plotted for sinusoidal stimulation of electrode pairs 1,2 and 3,4 at the varying depths of the IC. This example clearly demonstrates a distinct boundary between the central and external nuclei. Since each nucleus has its own cochleotopic organization, there are two distinct minima for each threshold function. The border

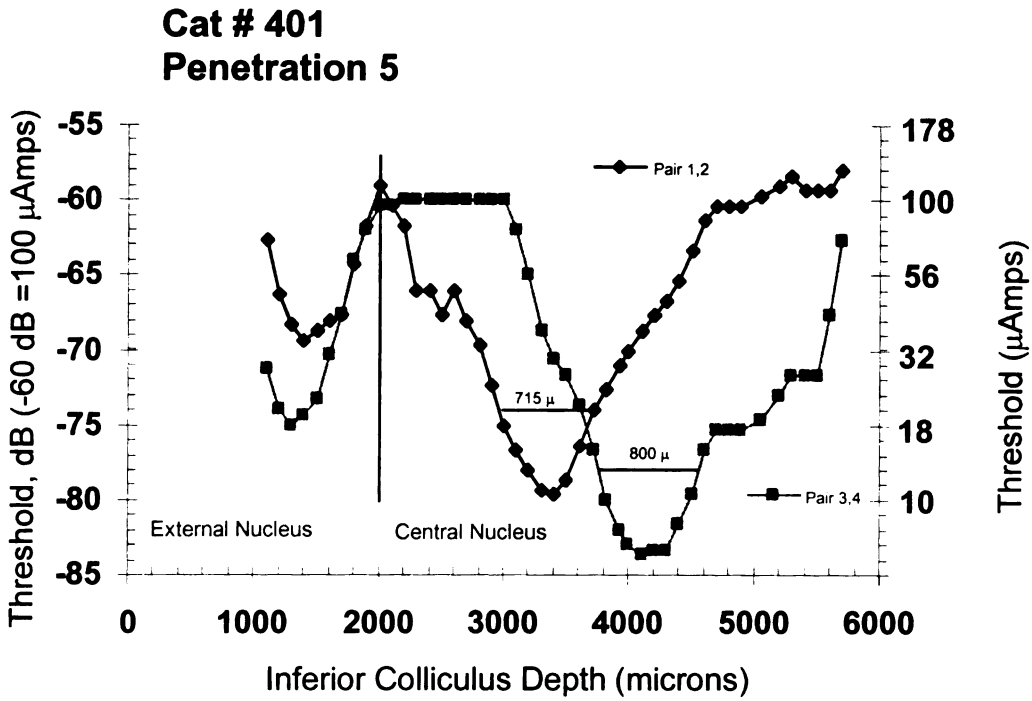
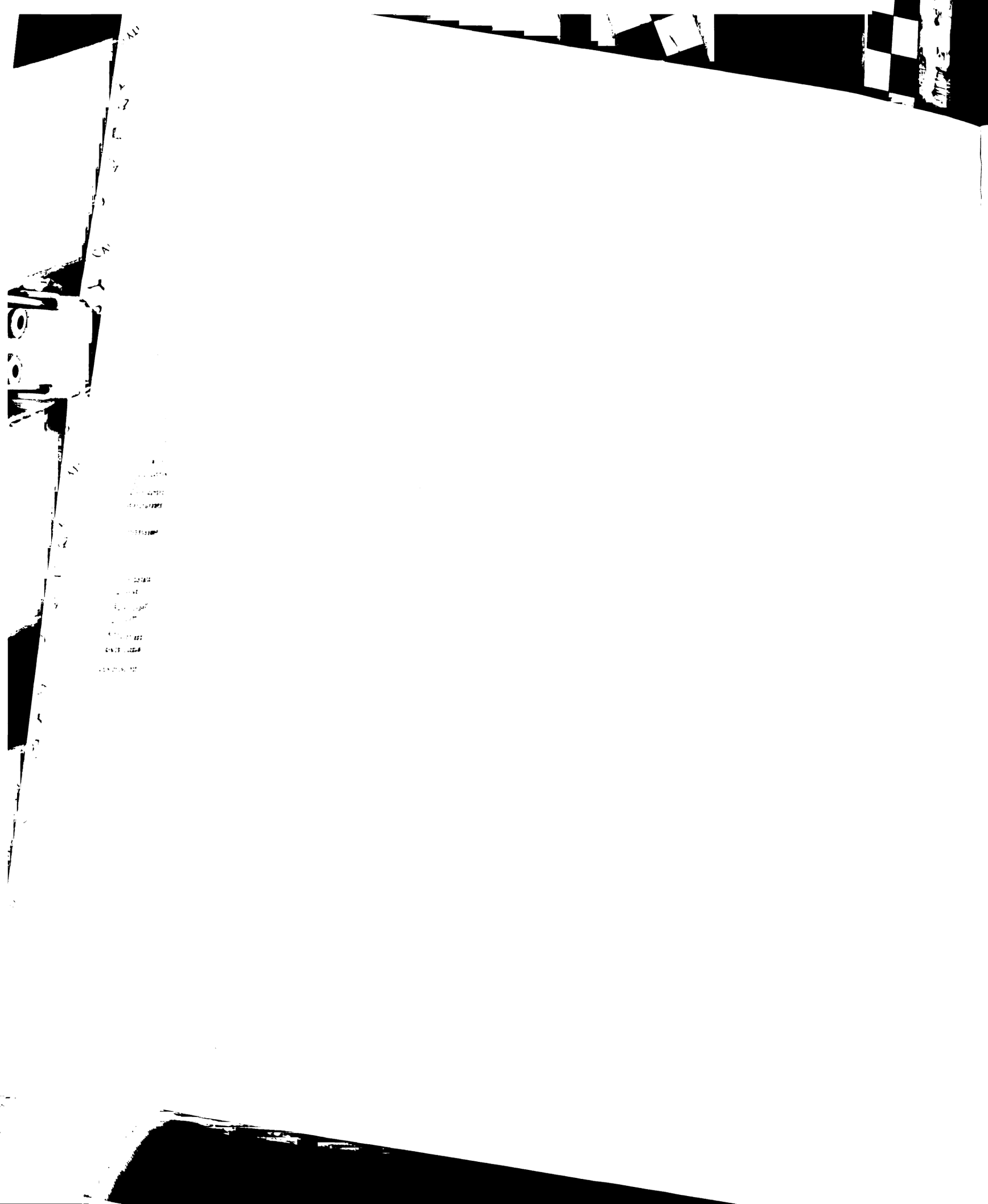


Figure 21. Exemplary spatial tuning curves (STC) recorded in the IC of a deaf cat. Responses thresholds are shown for an apical (1,2) and basal (3,4) stimulating electrode pair. Minimum threshold for the apical pair waslocated more superficially than the basal electrode pair. The widths of the STCs were measured at 6 dB above the minimum threshold, as indicated on each curve.

between the two nuclei is characterized by a high threshold region reflecting the relatively cell free region between them. This high threshold region was used to separate the single unit data by IC location (external and central nuclei).

As evident in **Figure 21** the threshold functions in the ICC can be described by their point of lowest threshold (minima) at a particular depth, width (microns) of IC activated at 6 dB above minimum threshold and dynamic range (minima to maximum thresholds obtained). With only one exception, the minimum threshold for the apical stimulating electrode pair was always positioned more superficially in the IC than the basal stimulating pair for all animals in every penetration. This corresponds to the systematic relationship of ICC depth and intracochlear electrode location (Snyder et al., 1990) and reflects the overall cochleotopic or frequency organization of the ICC. As previously described in acoustical studies, stimulation of low frequencies (apical regions of the cochlea) is represented more superficially in the ICC and progressively higher frequencies are represented deeper. Thus, depth for minimum threshold in the ICC gives an estimate of relative CF in a deafened animal. In this typical example, stimulation of the apical intracochlear electrode pair 1,2 resulted in a curve with a distinct minimum threshold (-81 dB) at 3400 microns from the surface of the IC. In contrast, stimulation of the basal (high frequency) electrode pair 3,4 had a lower minimum threshold (-84 dB) at 4100 microns.

As previously mentioned, the widths of the spatial tuning curves were measured at 6 dB above the minima to provide an estimate of the relative selectivity or spread of excitation across the cochleotopic (frequency) representation in deafened animals. In **figure 21**, the STC widths were very similar for stimulation of pair 1,2



and 3,4 at 715 and 800 microns, respectively. The dynamic ranges (the difference between the current level for minimum and maximum (saturation) threshold) were also similar for the two electrode pairs.

To demonstrate the variability typical in these data, **Figure 22abc** displays additional examples of spatial tuning curves recorded to sinusoidal stimulation and obtained from 3 different IC penetrations in one animal. The STCs are displayed for apical (1,2) and basal (3,4) stimulating electrode pairs and are labeled by penetration number. The 6 dB widths measured for each curve vary for each stimulating electrode pair and for all penetrations. For these 3 penetrations the STC width is always greater for pair 1,2 than 3,4. However, the difference in width between the 2 pairs varies from 40-1005 microns.

In order to facilitate comparisons among animals and electrode pairs, **Table 6** provides the mean tuning curve width calculated for each penetration recorded in every animal. For the majority of STCs a 6 dB width was easily determined. However, in a few instances incomplete data sets, unclear borders between the external and central nucleus, and/or the absence of a threshold minimum precluded accurate width calculations. Such data sets were not included in the averages of STC width.

The average 6 dB width data are presented in **Table 7**. In 3 of 4 cases the electrode pair 1,2 had an average 6 dB width greater than pair 3,4. However, these differences were not significant ($p=1.0$).

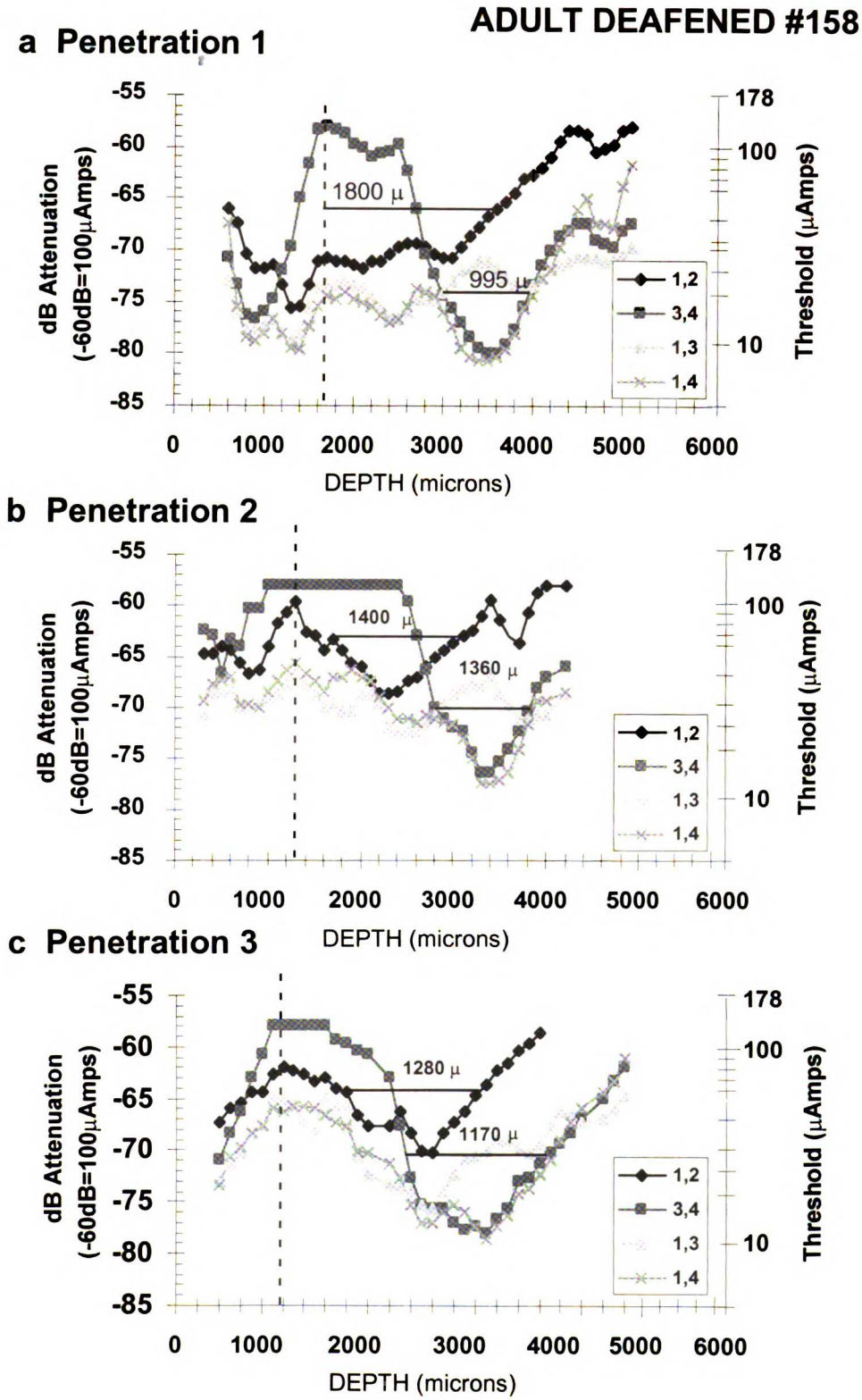


Figure 22abc. Spatial tuning curves from animal #158 for 3 different IC penetrations.

Animal	158		497		087		401	
Penetration	1,2	3,4	1,2	3,4	1,2	3,4	1,2	3,4
1	1800	995	Inc	Inc	1120	1180	760	620
2	1400	1360	1350	1200	880	1180	NoB	NoB
3	1280	1170	1260	540	540	1160	620	420
4	1200	900	NoB	NoB	1100	1310	500	Inc
5	1750	930	1420	1000	1500	1480	715	800
6			1000	1180				
7			900	1450				
average	1486	1106	1186	1074	1028	1262	649	613

Table 6. Spatial tuning curve dB widths and averages (microns) for pairs of electrodes recorded in 4 animals.

Inc=Incomplete data set; NoB=No discernable border

	Animal				
	158	497	401	087	average of all penetrations
Stimulating Pair					
1,2	1486	1186	649	1028	1087
3,4	1106	1074	613	1262	1014
1,4	1078	500	1115	1448	1034

Table 7. Average 6 dB widths (microns) for all penetrations of individual animals for each stimulating electrode pair. The average of all animals is provided at the right. There were no significant differences between the widths of the stimulating pairs (p=1.0).

3.2.4.1 Monopolar Sinusoidal Stimulation

Figure 23ab compares examples of tuning curves generated in the same penetration using bipolar and monopolar sinusoidal stimulation. The bipolar stimulation **(a)** shows distinct, although somewhat overlapping locations of stimulation. By comparison, the monopolar (M) stimulation **(b)** shows largely overlapping areas of excitation for the apical and basal electrodes. Stimulation of 1M and 2M produces minimum thresholds at similar depths, and these two electrodes have nearly identical 6 dB widths. Similarly, 3M and 4M have nearly identical widths and location of minimum threshold at similar depths. In this particular example, the results suggest that the most basal electrodes (3M and 4M) stimulate neural populations that have marked overlap with, and essentially comprise a subset of the population stimulated by apical monopolar electrodes 1M and 2M. In general, however, apical electrodes tend to activate distinct populations from the basal electrodes.

Overall, monopolar stimulation produced tuning curves with considerable variability between the 4 intracochlear electrodes and more variability than bipolar stimulation. Thresholds, tuning curve shape and spatial selectivity all varied widely within and between animals. However, the STCs generated from monopolar stimulation in many cases did provide valuable information for defining the border between the external and central nucleus.

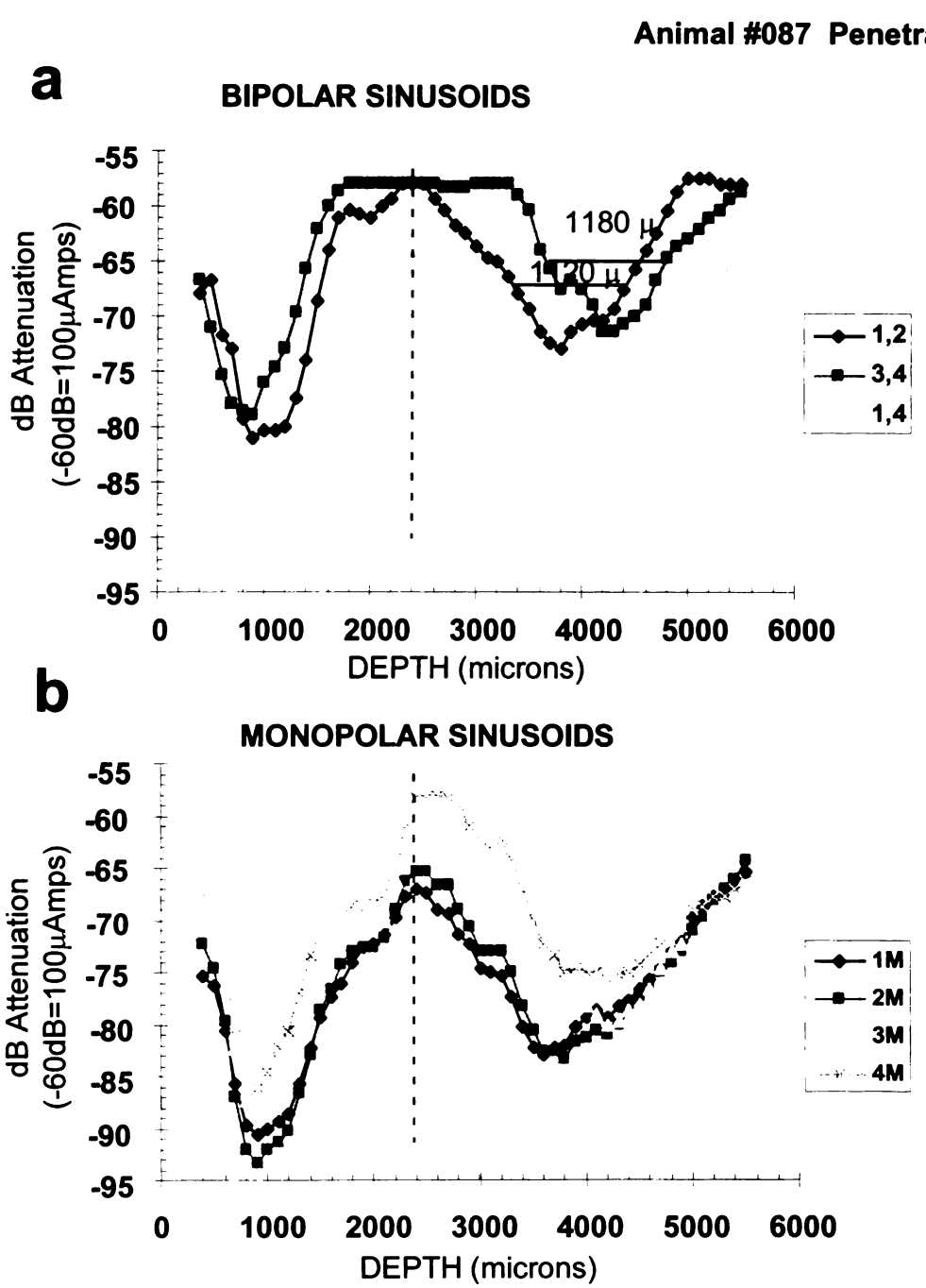


Figure 23ab. STCs to monopolar and bipolar sinusoidal stimulation for the same penetration in one animal. This example illustrates a clear border between external and central nuclei.

3.2.4.2 STCs Pulsatile Stimulation

Since chronic electrical stimulation signals were comprised of biphasic pulses it was important to examine the responses of IC neurons to pulsatile stimulation. Further, because chronic stimulation levels were set based on the EABR response threshold to pulsatile stimulation a comparison of these thresholds to IC responses obtained with the same signal provides information as to the spread of excitation within the IC in response to the peripheral stimulation.

A comparison of IC minimum thresholds to the EABR thresholds for the individual animals is plotted in **figure 24.** The fairly high Pearson's correlation ($r = 0.64$), suggests that the EABR may co-vary with minimum single unit IC threshold.

In order to estimate the area within the IC activated by the chronic stimulation, spatial tuning curves were constructed from IC thresholds obtained at 100 micron intervals in response to pulses presented to electrode pair 1,2. **Figures 25 and 26** show examples of STCs recorded in 2 animals. **Figure 25** shows the similarity among 4 electrode penetrations for pulsatile stimulation of the apical electrode pair (1,2) in animal #401. All penetrations show similar patterns of response thresholds for the varying depths of the IC. In this example, the border between the external and central nucleus is clearly evident at approximately 1600 microns in all penetrations. The horizontal dashed line denotes the current level of daily chronic stimulation, showing that approximately one half of the ICC was activated during daily stimulation. **Figure 26** is a plot of response threshold as a function of IC depth for 4 separate penetrations

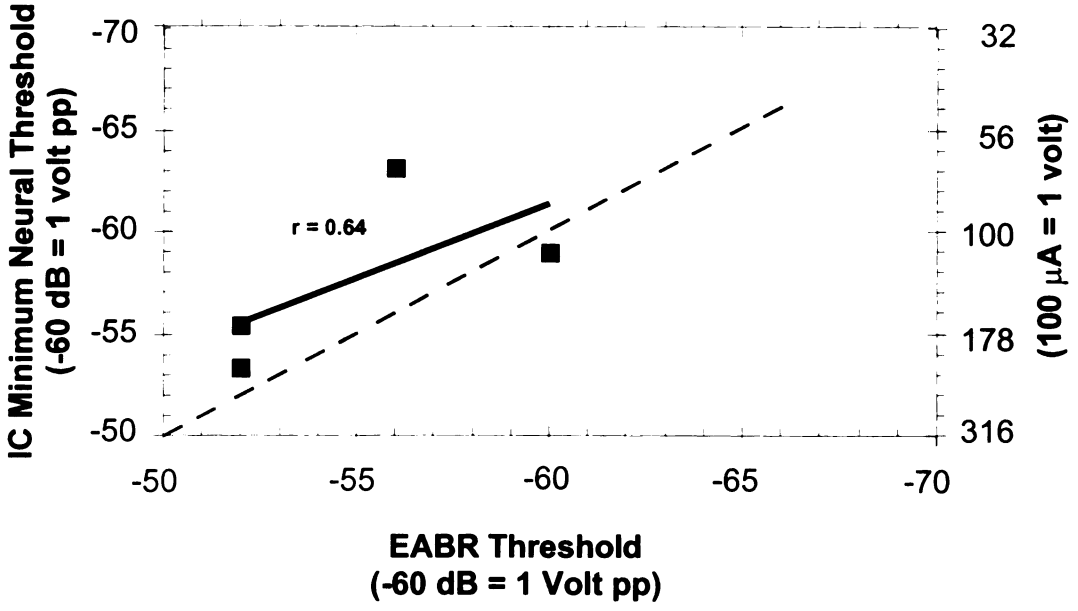


Figure 24. Comparison of the minimum (best) IC neural threshold to the final EABR threshold for each individual animal. Pearson's coefficient suggests a postive correlation (0.64) between these two measures.

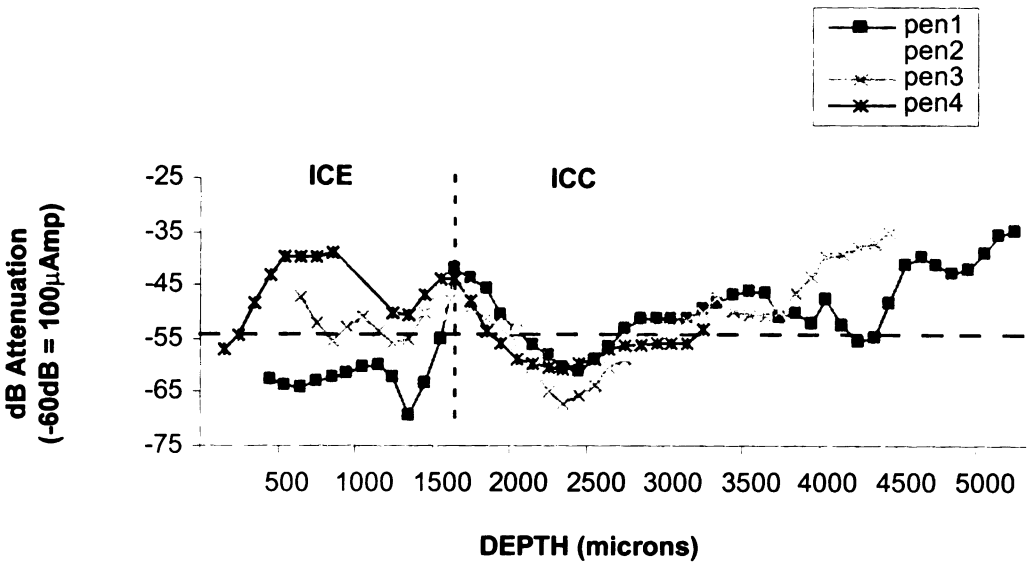


Figure 25. Spatial tuning curves for bipolar pulses presented to pair 1,2 for 4 penetrations obtained from animal #401. Vertical dashed line denotes border between ICE and ICC. Horizontal dashed line at final current level for chronic stimulation.

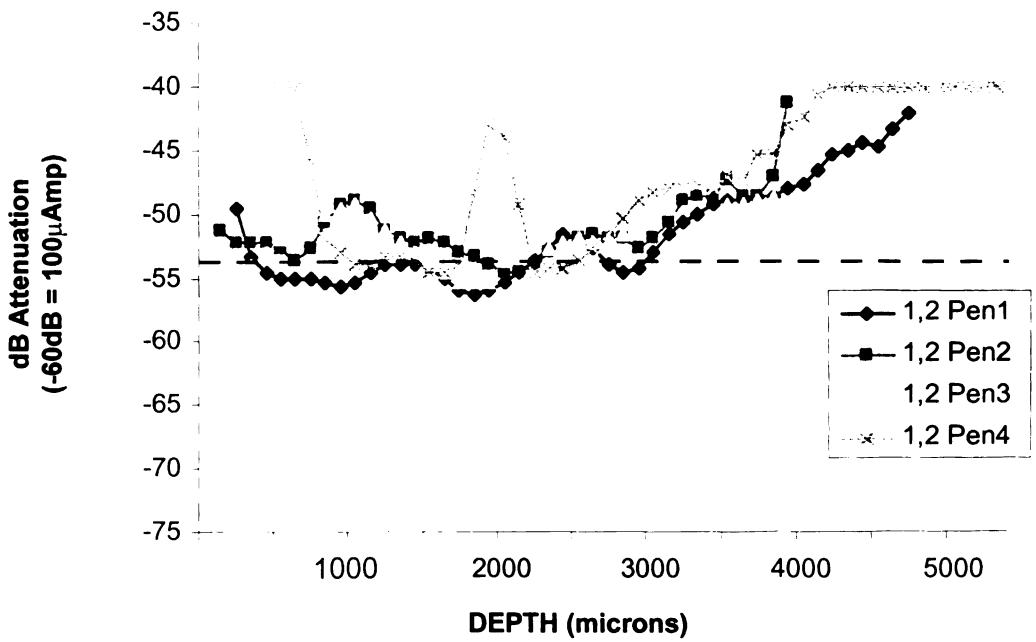


Figure 26. Spatial tuning curves for bipolar pulses on pair 1,2, constructed from data obtained from 4 penetrations in animal #158. No border between the ICE and ICC could be determined. Dashed line denotes final chronic stimulation level.

obtained in animal #158. In this example the border between ICE and ICC is not evident and thus it had to be determined from the sinusoidal STC data. Again, the horizontal dashed line indicates the current level of chronic electrical stimulation.

Figure 27abc are examples of spatial tuning curves recorded from animal #497 to pulsatile stimulation. In these examples response thresholds are plotted as a function of IC depth in response to bipolar stimulation of pairs 1,2 and 3,4 for three different electrode penetrations. The horizontal dashed line shows the daily chronic stimulation level for this animal. These results demonstrate the marked variability that is more commonly observed with pulsatile stimulation than with sinusoidal stimulation. In **Figure 27a**, electrode pair 1,2 has a response minimum at a more superficial region of the IC, whereas electrode pair 3,4 has its minimum at a deeper level reflecting the normal frequency organization of the ICC. These functions would suggest that electrode pairs 1,2 and 3,4 stimulate completely distinct populations of neurons at 2 dB above minimum threshold. In the next set of graphs, (b and c), although the curves are not offset in the same orderly fashion, they also do appear to show that the apical and basal channels stimulate different regions of the IC. For example, in **figure 27b**, note that stimulation at 2 dB above the EABR threshold (–58 dB) for pair 1,2 would activate neurons located about 2200-2300 and 3100-3500 μm, a different population of neurons than the population activated by electrode pair 3,4 (2800-3100 μm). Moreover, there are differences in the response thresholds for stimulation between the two pairs in one penetration pair 1,2 has the lower threshold (see **Figure 27b**), whereas, the situation is reversed in the next penetration with pair

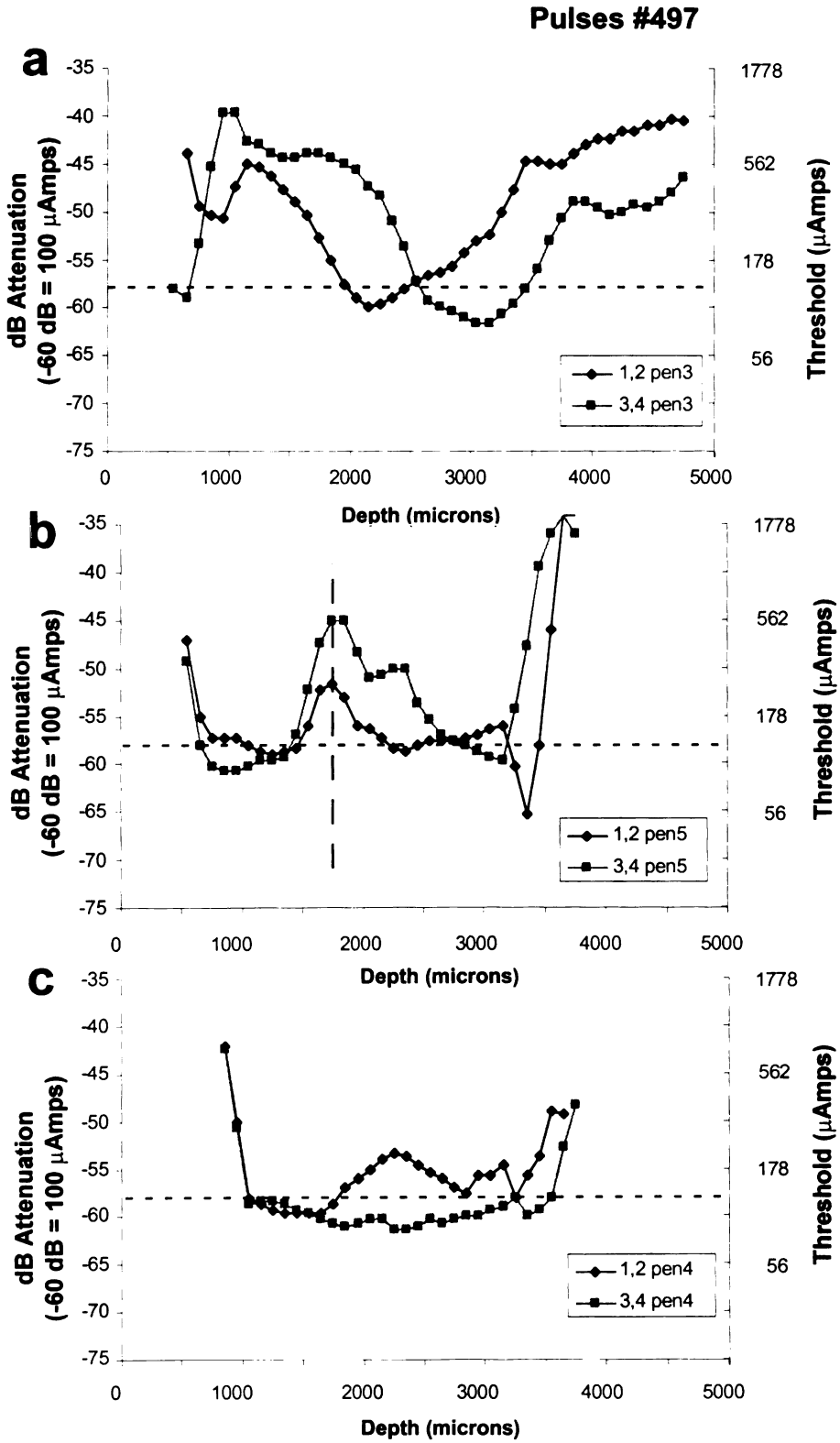


Figure 27abc. Response thresholds to bipolar pulses are plotted as a function of IC depth for 3 different penetrations (a-c) from animal #497. Dashed horizontal line denotes daily chronic electrical stimulation level. The vertical dashed line in 27b shows the border between the external and central nucleus.

3,4 having the lower threshold. Further, in these examples, the border between the central and external nucleus is evident only in penetration 5 (**Figure 27b**).

3.2.4.3 Pulsatile vs Sinusoidal: Threshold, dynamic range and STC width

It is important to consider differences in the response of IC neurons to varied stimulus waveforms. Comparison of STCs evoked by pulsatile (0.2 ms/phase) and 3 cycles of a 100 Hz sinusoid reveal a number of contrasts. In general, the pulsatile stimuli (0.2ms/phase) give higher response thresholds, reduced dynamic ranges (in dB), and broader spatial tuning curve widths than the longer phase duration sinusoidal stimuli. However, as will be demonstrated, the dynamic range for pulses appears to be compressed when compared to sinusoidal responses only when values are expressed in dB; when expressed in absolute current levels the dynamic range for pulses greatly exceeds the dynamic range of sinusoids.

Figure 28 and 29 show STCs constructed for 2 different penetrations in one experimental animal. In both figures response thresholds are plotted as a function of depth for the stimulation of electrode pair 1,2 (dark symbols) and 3,4 (light symbols). STCs are shown for pulsatile (**a**) and sinusoidal stimulation (**b**).

For *pulsatile* stimulation (**Figure 28a** and **Figure 29a**) the minimum response thresholds (ICC) were similar in both penetrations for both electrode pairs at approximately –55 dB (178 μA). However, the dynamic ranges (maximum threshold minus minimum threshold) varied between the two penetrations and the different electrode pairs. In **figure 28a** (penetration #2) the dynamic range was approximately 13 dB (530 μA) for pair 1,2 and 18 dB (1234 μA) for pair 3,4. In **Figure 29a**

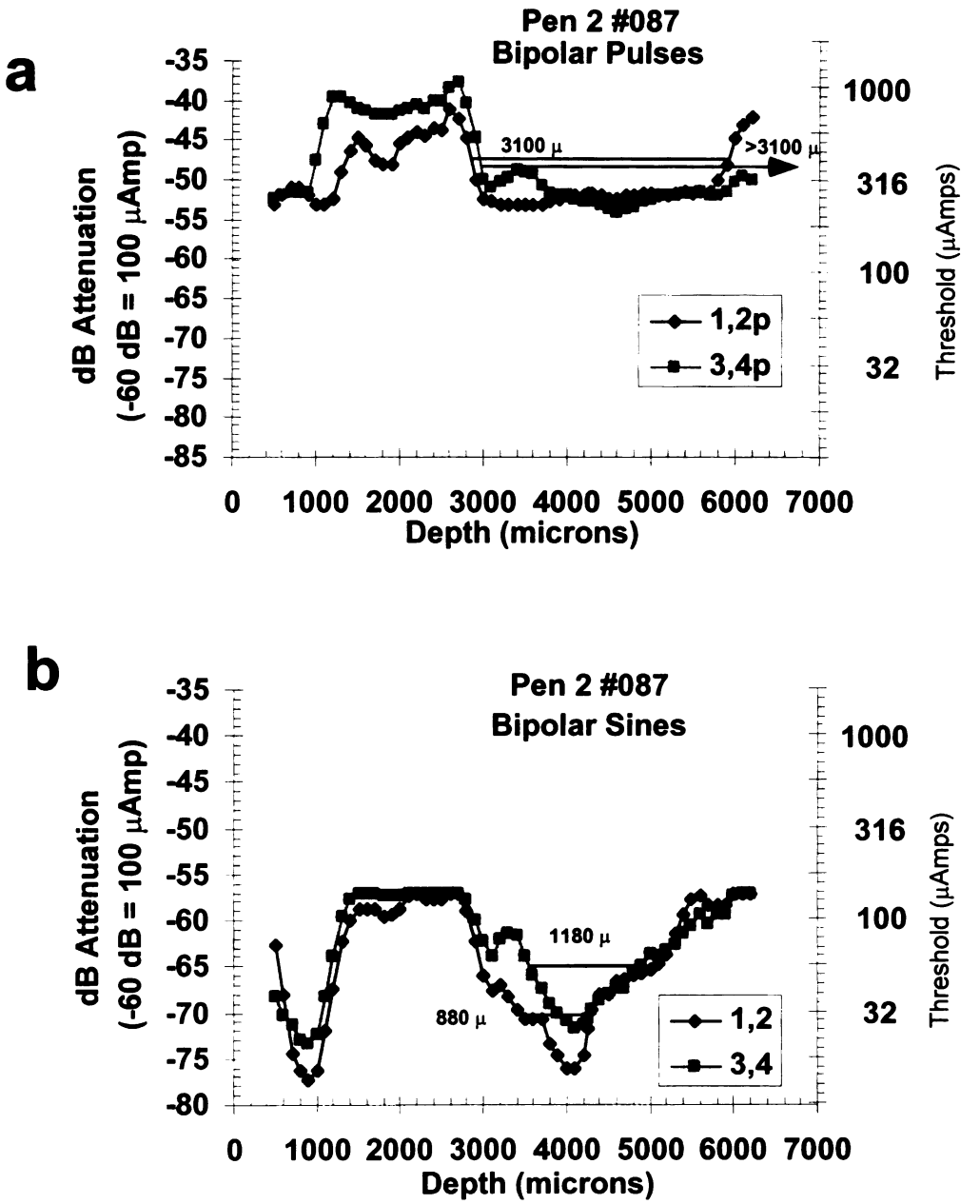


Figure 28ab. Examples of STCs obtained from penetration 2 in animal #087. Response thresholds to pulsatile stimulation (a) and sinusoidal stimulation(b) for two electrode combinations.

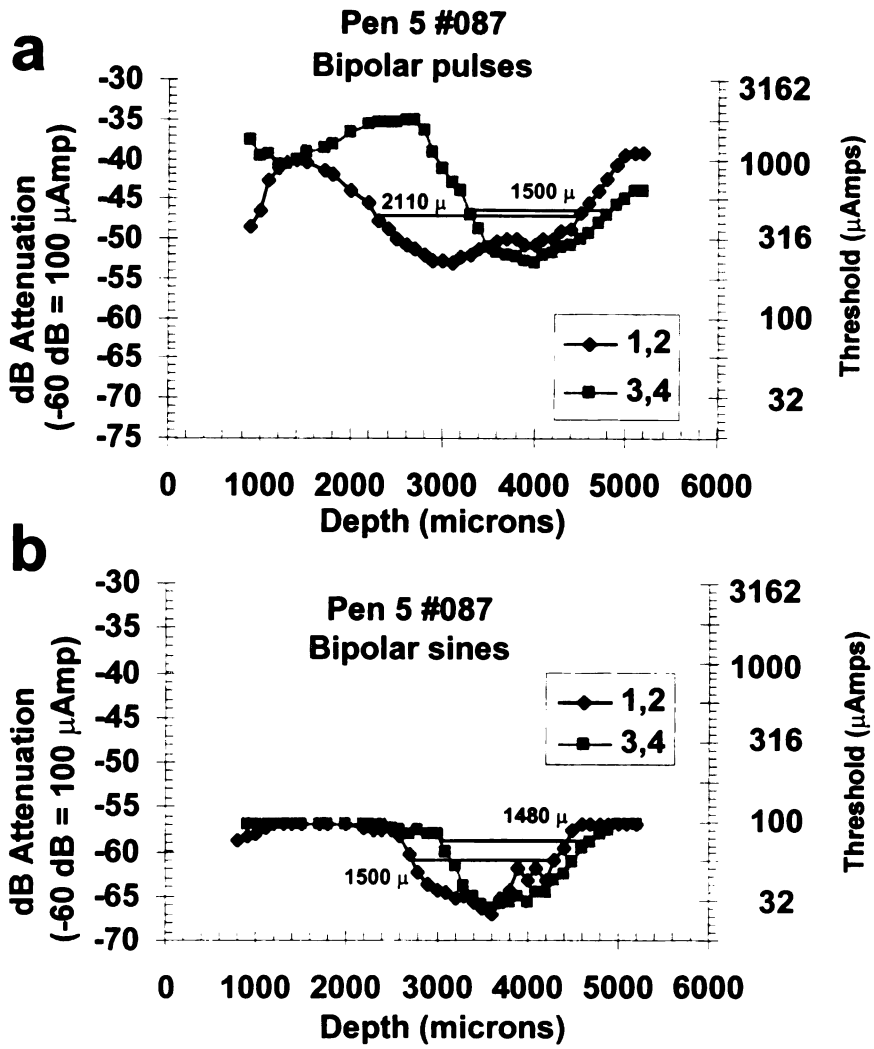


Figure 29ab. Examples recorded from one penetration in animal #087. Responses to 200 µs/phase pulses (top) and responses to sinusoidal stimulation (bottom) for two electrode pairs. Black horizontal lines show 6 dB width.

(penetration 5) the dynamic ranges were nearly equal for the 2 electrode pairs, -16 dB (1059 μA) and –17 dB (1527 μA) for pair 1,2 and 3,4 respectively.

The spatial tuning curve widths for pulses measured at 6 dB above response minima also differed for the 2 penetrations and electrode combinations. In penetration #2, **(Figure 28a)** the 6 dB width was 3100 μ for pair 1,2 and ≥3100 μ for pair 3,4. In penetration #5 the STC widths were narrower suggesting relatively more discrete activation of the IC at this location **(Figure 29a)**. For this penetration the STC widths were 2100 μ for pair 1,2 and 1500 μ for pair 3,4.

In contrast, STCs for *sinusoidal* stimulation **(figure 28b)** show much lower response thresholds, greater dynamic range (in dB) and narrower 6 dB widths than for pulsatile stimulation. Additionally, between penetrations there was considerable difference in threshold minima, dynamic range and STC width. **Figure 28b** (penetration #2) has a threshold minima of -77 dB (14 μA) for pair 1,2 and -72 dB (25 μA) for pair 3,4. In penetration #5 **(Figure 29b)** the thresholds were somewhat higher, with a minima of –68 dB (40 μA) and –65 dB (56 μA) for pair 1,2 and 3,4, respectively. Dynamic ranges were comparable for the two penetrations. Dynamic ranges in penetration #2 (Figure 28b) were 19 dB for both electrode pairs (127 and 199 μA for pair 1,2 and 3,4 respectively) and in penetration #5 (Figure 29b) the dynamic ranges were 11 dB (100 μA) for pair 1,2 and 8dB (85 μA) for pair 3,4.

The STC widths for sinusoidal stimulation in both penetrations were consistent with much more restricted activation of the IC than that observed with pulsatile stimulation. In **Figure 28b** (penetration #2) the 6 dB widths were 880 μ for pair 1,2

and 1180 μ for pair 3,4. In **Figure 29b** the STCs were 1500 and 1480 μ for pair 1,2 and 3,4 respectively.

These examples were chosen to illustrate the characteristic differences between sinusoidal and pulsatile stimuli and further, to illustrate the variability which can be recorded at different IC locations. Sinusoidal stimulation had consistently lower thresholds and narrower 6 dB STC widths than pulsatile stimulation. In addition, the dynamic ranges were greater for sinusoidal stimulation when calculated in dB; however, when calculated in absolute current, dynamic range was always *reduced* for sinusoidal stimulation as compared to pulsatile stimulation. These findings are not surprising given the difference in phase duration of each signal. That is, a 100 Hz sinusoid has a 5ms/phase duration, roughly 25 times that of the 200 μsec/phase pulses.

3.3 Cochlear Morphology

Following the final acute electrophysiology experiments the cochleas were preserved, histological studies were conducted, and morphometric data were collected to assess neural survival. Data were obtained from all animals receiving chronic stimulation including 507 and 403 for which final electrophysiological data were not available. The summary of these stimulation histories are presented in **Table 8.**

The animals were divided into two groups post hoc based on length of stimulation and condition of the cochlea. **Group 1** includes one animal (#403) that did not meet the criterion of 24 weeks of stimulation and two animals with severe intractable infections at the percutaneous connector (#507 and #158). Because of the short duration of

Animal	Stimulation Period (days/wks)	Level (μAmp)	Duration Deafness (weeks)
ANIMALS WITH SHORTER STIMULATION PERIODS AND/OR SEVERE COCHLEAR PATHOLOGY			
403	66 /11	50-79	13
507	70/20	100-200	22
158	116/22	100	24
ANIMALS COMPLETING STIMULATION			
497	133/30	50-126	32
087	144/32	100-251	33
401	105/21	126-200	23

Table 8. Stimulation histories for animals included in this study.

stimulation in the first case and the severe cochlear pathology observed in the other 2 animals, the cochlear data from this first group are considered separately.

Group 2 is comprised of 3 animals that successfully completed the stimulation period without apparent problems.

3.3.1 Locations of Intracochlear Electrodes

The locations of electrode contacts were determined in the fixed temporal bones (see Methods, section 2.62). With the electrode in situ, the scala vestibuli of the basal turn was opened, the metallic electrode contacts were visualized, and their positions marked in the bone adjacent to the stria. These markers were preserved in the surface preparation and used to determine the location of each electrode.

Table 9 provides the location of each electrode in mm and in percent distance from cochlear base. The frequency represented at the mean location of each electrode was then calculated using the Greenwood (1974) frequency-position function with the revised constants obtained by Liberman for best fitting the Greenwood function to data for the cat (Liberman, 1982). The average position of electrode #1 was 12.8 mm, or 4.5 kHz and for electrode #2 it was 11.6 mm or 5.8 kHz. Thus, the stimulating (apical) electrode pair 1,2 was centered at the cochlear region representing about 5.2 kHz. The basal electrodes 3 and 4 had average locations of 7.6 mm and 6.6 mm, representing frequencies of 13.1 and 15.9 kHz, respectively. Thus, the basal channel in these cats was centered near 14.5 kHz.

	Electrode Contact				
	1	2	3	4	Cochlea Length (mm)
	position/distance from cochlear base (mm/%)				
Animal					
087	**12.3 (49)**	**11.2 (44.8)**	**7.1 (28.0)**	**6.1 (24.4)**	**25.0**
497	**12.5 (50)**	**11.5 (46.0)**	**7.5 (30.0)**	**6.5 (26.0)**	**25.0**
401	**12.1 (48)**	**11.2 (44.4)**	**7.2 (28.5)**	**6.2 (24.6)**	**25.2**
158	**13.5 (56)**	**11.5 (48.0)**	**7.5 (31.1)**	**6.5 (26.9)**	**24.1**
403	**13.7 (51.5)**	**12.7 (47.4)**	**8.7 (32.5)**	**7.7 (28.7)**	**26.8**
507*	na	na	na		**25.5**
Mean	**12.8 (50.9%)**	**11.62 (46.2%)**	**7.6 (30.0%)**	**6.6(25.8%)**	**25.3 mm**
Frequency	**4.51 kHz**	**5.8 kHz**	**13.1 kHz**	**15.9 kHz**	

Table 9. The location of the stimulating electrode contacts (#1-4) in mm from cochlear base and percent distance along the cochlear spiral are given. Cochlear length (mm) is provided for all animals. (*/na denotes case where electrode contacts could not be visualized.) The mean distance from the cochlear base for each electrode contact and the mean estimated frequency represented by each electrode is provided (see text).

3.3.2 Group 1

The first animal in this group died after only 11 weeks of chronic stimulation when it was sedated for repair of its implant connector. Therefore, it did not meet the criterion for length of chronic stimulation. Examination of the implanted cochleae of the animals with chronic infection (#507 and #158) revealed severe pathology. **Figures 30a and b** show representative histological sections documenting the cochlear pathology in these specimens. **Figure 30a** shows a region 9.5 mm from the cochlear base in cat 158, illustrating severe labyrinthitis and trauma resulting from insertion of the implant. Extensive neovasculature, chronic inflammation and the ruptured basilar membrane are evident. Animal #507 damaged its implant and required reimplantation after approximately 12 weeks of stimulation. During the reimplantation surgery the scala tymani was found to be occluded by polypoid-like tissue which made insertion of a new electrode difficult. The round window was fractured during an attempt to remove this tissue from the scala, and the resulting pathology was severe and extended throughout the cochlea. **Figure 30b** shows a section taken from the basal region of animal #507. The pathology consequent from labyrinthitis and temporal bone fracture in these animals was considered so severe that it was likely to have compromised the spiral ganglion cell survival in these cases. Thus, their results in regard to effects of chronic stimulation in neural survival were considered invalid. Morphometric data were collected from these animals, but they were considered as a separate group along with the animals with a short stimulation period.

The individual morphometric data for these first 3 cases are presented in **Figure 31abc**. SGC density is plotted as percent of normal in 10% intervals from base to apex

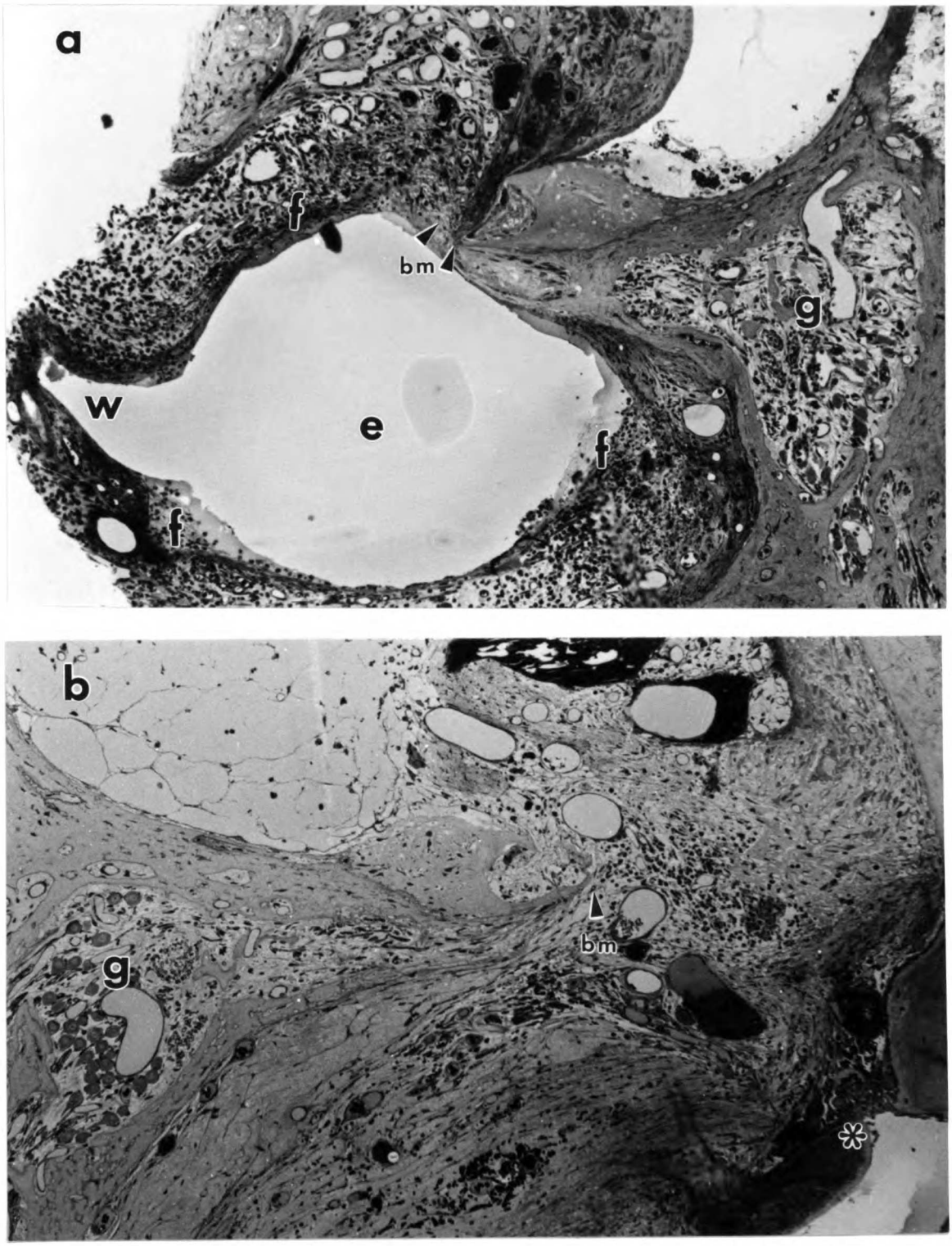


Figure 30ab. a) Photomicrographs of a section taken from the 9.5 mm region of animal #158. Note the profile of the implanted electrode (e); including the 'wing' (w) outline at the left within the highly reactive fibrotic (f) connective tissue encapsulating the implant. The high density of polymorphonuclearleucocytes, monocytes infiltrating this tissue is evidence of chronic labyrinthitis. Insertion trauma has resulted in severe basilar membrane (bm) rupture. b) Photomicrograph taken of a section from the 3.5 mm region of animal #507. This animal required reimplantation due to device failure. During surgery the round window was fractured (*) and the basilar memebrane (bm) ruptured severely. Chronic infection and severe inflammation were evident. These cochlea were deleted from the study due to this severe pathology which was judged to effect SGC (g) survival.

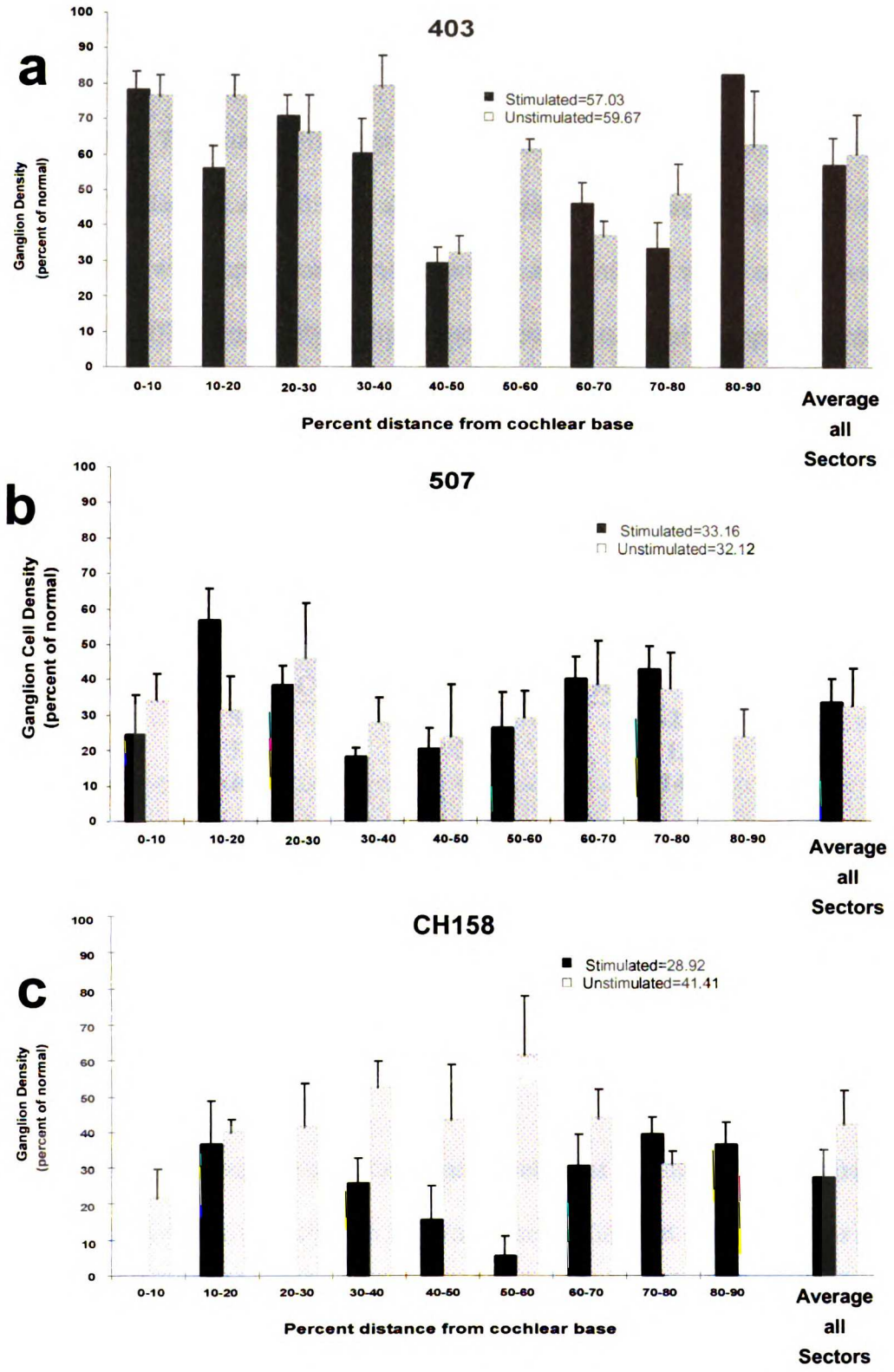


Figure 31abc. Individual morphometric data for Group 1 animals. Error bars indicate standard deviation from the mean.

of the cochlea. The results from the stimulated and unstimulated cochleae are compared for each sector. The final data bars indicate the overall averages across all sectors. The quantitative data for the individual animals are given in **Table 10**. The SGC data for animal #403 show symmetrical and relatively good survival bilaterally with approximately 60% survival after only 13 weeks of deafness. Animal #507 (chronic labyrinthitis) had an overall survival of approximately 32% in both ears. The unstimulated ear SGC survival was comparable to that observed for the other control

ANIMAL	Stimulated	Unstimulated	Difference (Stim-Unstim)
ANIMALS WITH SHORTER STIMULATION PERIODS AND/OR SEVERE COCHLEAR PATHOLOGY			
403	57.03	59.67	-2.64
507	33.16	32.12	1.04
158	26.99	41.41	-14.42
ANIMALS COMPLETING STIMULATION			
497	53.44	36.84	16.6
087	52.63	43.72	8.91
401	38.15	33.94	4.21

Table 10. Spiral ganglion cell survival for the stimulated and unstimulated cochlea of all animals.

ears in this study whereas the stimulated ear had obvious pathology (see **Figure 30b**) and chronic labyrinthitis which even infiltrated the modiolus at some locations and compromised SGC survival. The implanted cochlea from #158 was so severely compromised (fibrous tissue growth) that two basal regions were lost from the surface preparation and could not be examined. The remaining basal and mid cochlear sectors had severe ganglion cell loss.

3.3.3 Group 2

Animals included in Group 2 did not have obvious evidence of pathology and met the chronic stimulation period criterion specified by the experimental protocol. **Figure 32ab** show examples taken from an apical (a) and basal (b) sector of a deafened control cochlea. There were no surviving hair cells in any of the control or stimulated ears in the study. In the example shown in Figure 32a the pillar cells have collapsed and the supporting cells cannot be differentiated. In figure 32b taken from the apical region there is better survival of supporting cells. The tunnel of Corti is still visible, but the lateral pillar cell is on the verge of collapse. The individual morphometric SGC data for the animals in group 2 are presented in **Figure 33abc.** Again, SGC density is plotted for 10% intervals from base to apex and shown as percent of normal for each cochlear sector. Data are compared for the stimulated and unstimulated cochleas, and the average for each ear is shown at the right of each graph. The overall quantitative data are presented in **Table 10.**

Careful examination of the data in the control, unstimulated cochleas **(Figure 33)** reveals a variable and patchy pattern of SGC loss in the different sectors of the individual cochleas. However, in almost every cochlear sector, SGC density was greater in the cochlea that received chronic electrical stimulation than in the paired data from the unstimulated ear **(Figure 33abc)**. A striking exception to this rule was seen in the 40-50% sector, where all the implanted cochleas exhibited damage (*) from the insertion trauma caused by the tip of the electrode. **Figure 34** shows a histological section taken from this 40-50% region in # 087, illustrating a typical fracture of the osseous spiral lamina near the electrode tip. This trauma clearly resulted in reduced SGC survival and

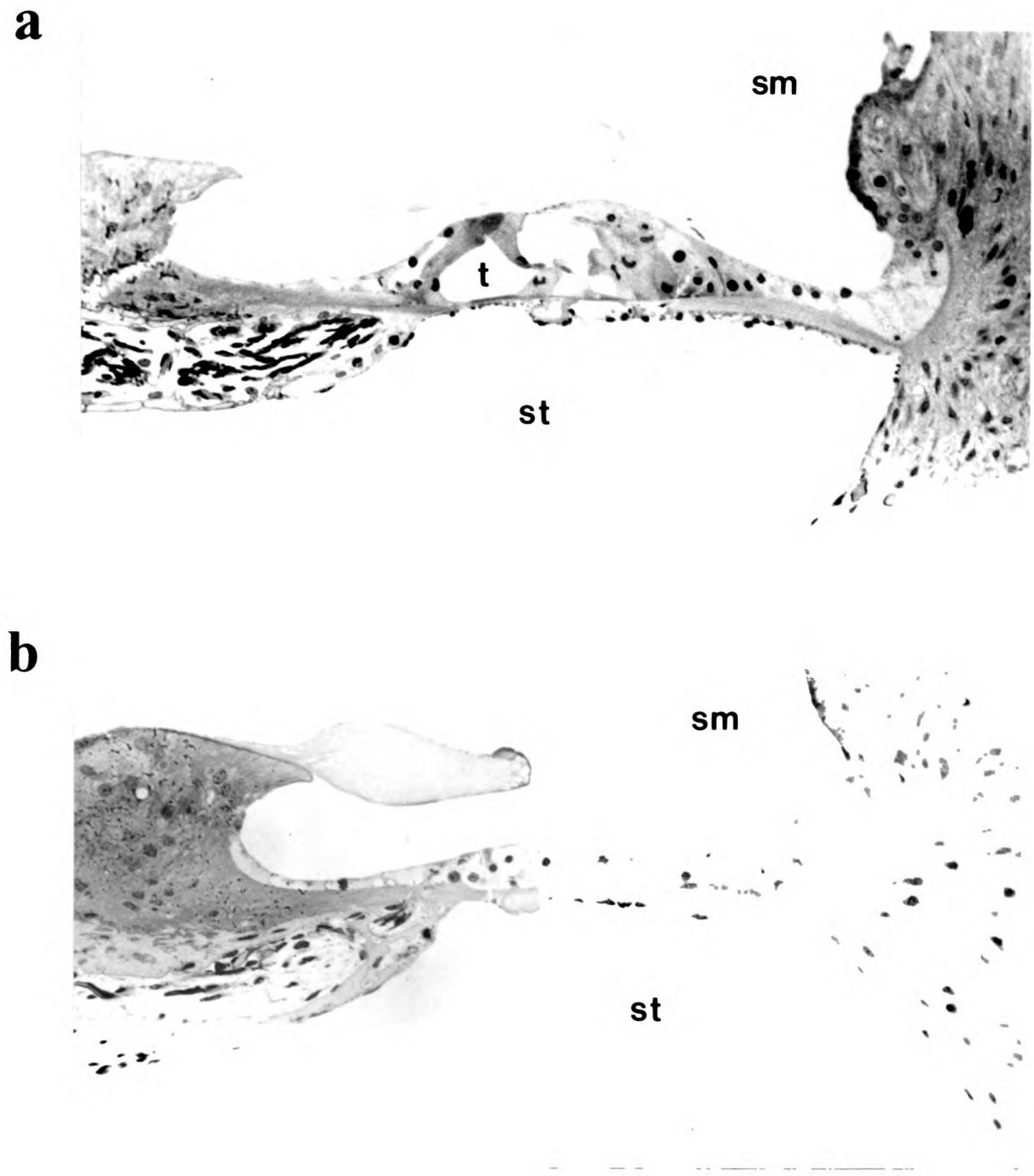


Figure 32ab. Photomicrographs of representative histological sections from the control, deafened cochlear of animal #497. Top photo (a) shows an apical section taken approximately (19.5 mm; 70-80% region) from the base. Note the presence of the tunnel of Corti with damaged pillar cell. Photo (b) taken from the base (5.0 mm; 20-30% region) shows no tunnel of Corti and undifferentiated supporting cells. (sm = scala media; st = scala tympani; t = tunnel of Corti)

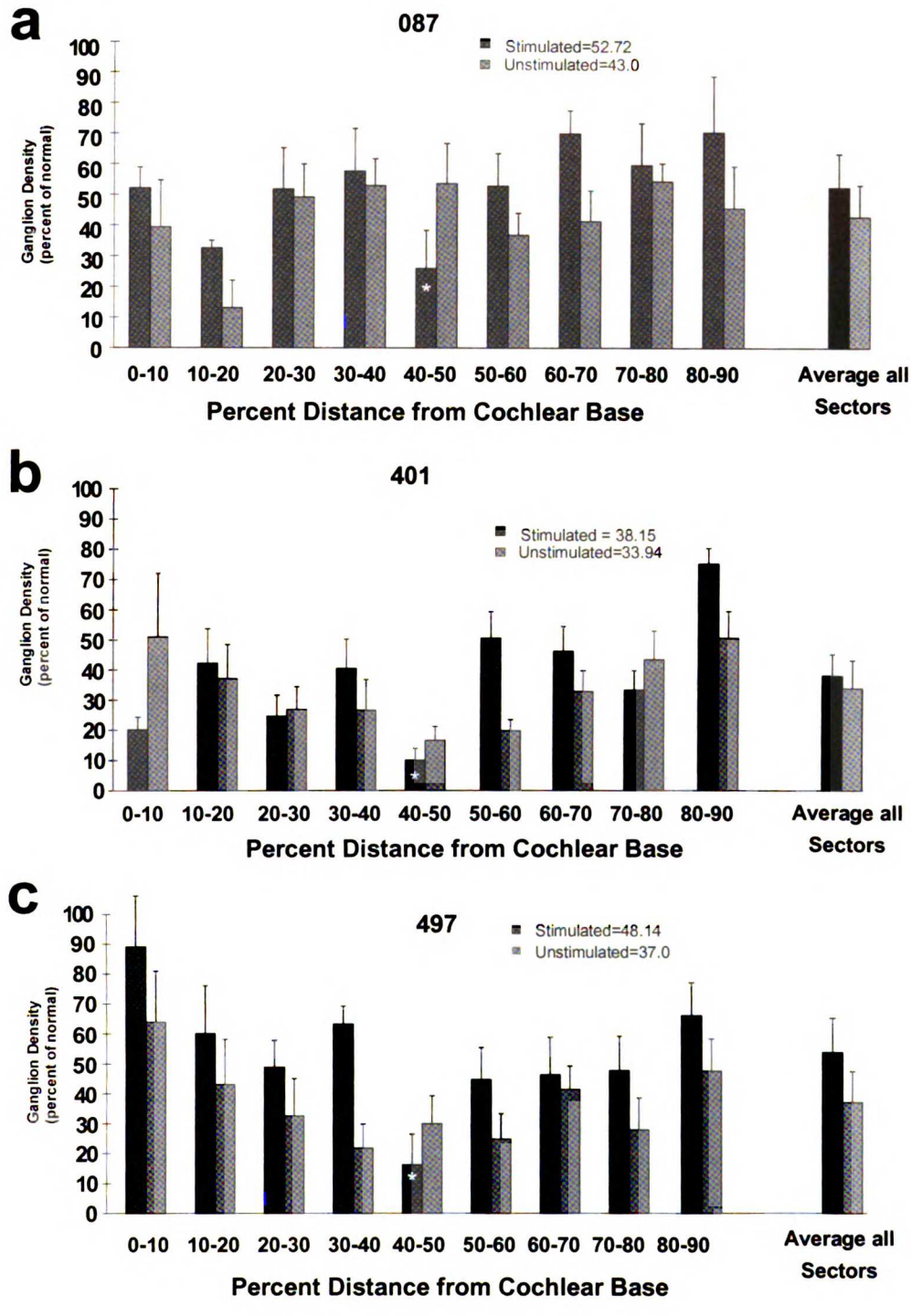


Figure 33abc. Individual morphometric data illustrating SGC survival in Group 2 animals. Data are presented as percent of normal for the stimulated and unstimulated cochlea. Error bars indicate standard deviation from the mean.

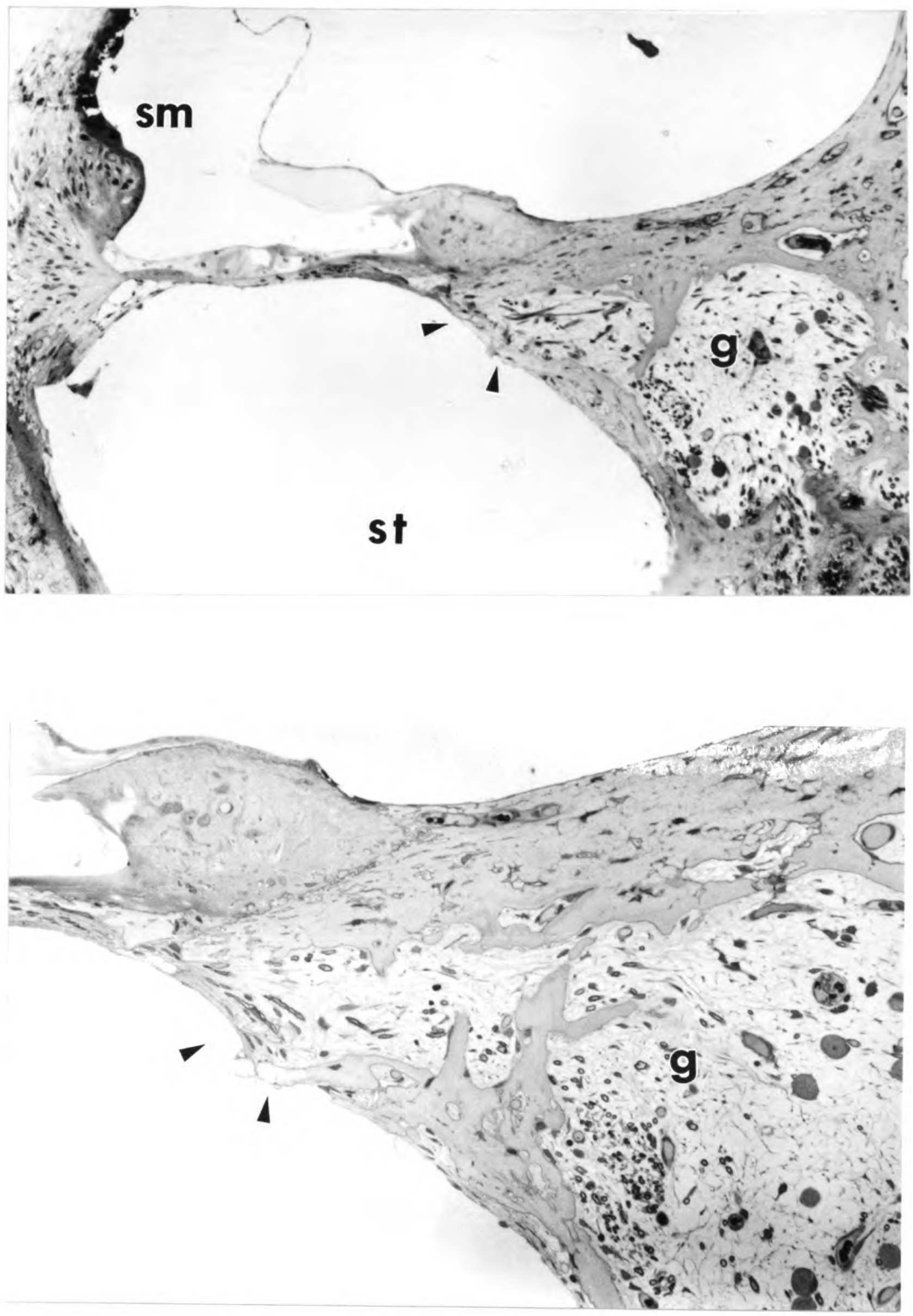


Figure 34. Photomicrographs of histological sections taken from the 11.5 mm (40-50%) region in Animal #497 showing the fractured (noted by arrows) osseous spiral lamina. This trauma from electrode insertion results in extensive sprial ganglion cell (g) loss in this region.

extensive cell loss. In broken serial sections through such regions it was apparent that the extent of neural degeneration corresponded to the degree of damage incurred.

Figure 35 presents the average SGC density pooled for this group of 3 cats, again shown as percent of normal for each cochlear sector in the stimulated and unstimulated ears. Each sector of the cochlea (except the 40-50% area with electrode tip damage) had higher SG cell survival in the stimulated ear as compared to the control side. Although the small *n* in this group completing chronic stimulation precluded statistical analyses, mean SGC density for all cochlear sectors, was 10% higher in the stimulated cochleae (48.10% of normal) than that in the paired, unstimulated ears (37.95% of normal).

The *difference* in SG density between stimulated and unstimulated ears is plotted in **Figure 36**. Data are again expressed as percent of normal for each cochlear sector from base to apex. The obvious effect of insertion trauma at the tip of the electrode is shown by the negative value in the 40-50% region. All other cochlear regions showed increases in ganglion cell survival as a consequence of chronic stimulation. It is interesting to note that the greatest effect was seen in the regions immediately basal and apical to the stimulating electrodes (i.e., 30-40% and 50-60%), although a comparable increase is also observed in the most apical sector. Again, averaged over all cochlear sectors, 10% of the normal cell density is maintained in the stimulated cochleae over that in the paired unstimulated ears. **Figure 37ab** shows photomicrographs of sections taken from the 13 mm region comparing the SGC densities in the stimulated ear (**a**) and unstimulated ear (**b**). The increased survival is visible for the stimulated (44.6%) ear as compared to the unstimulated (24.7%) ear.

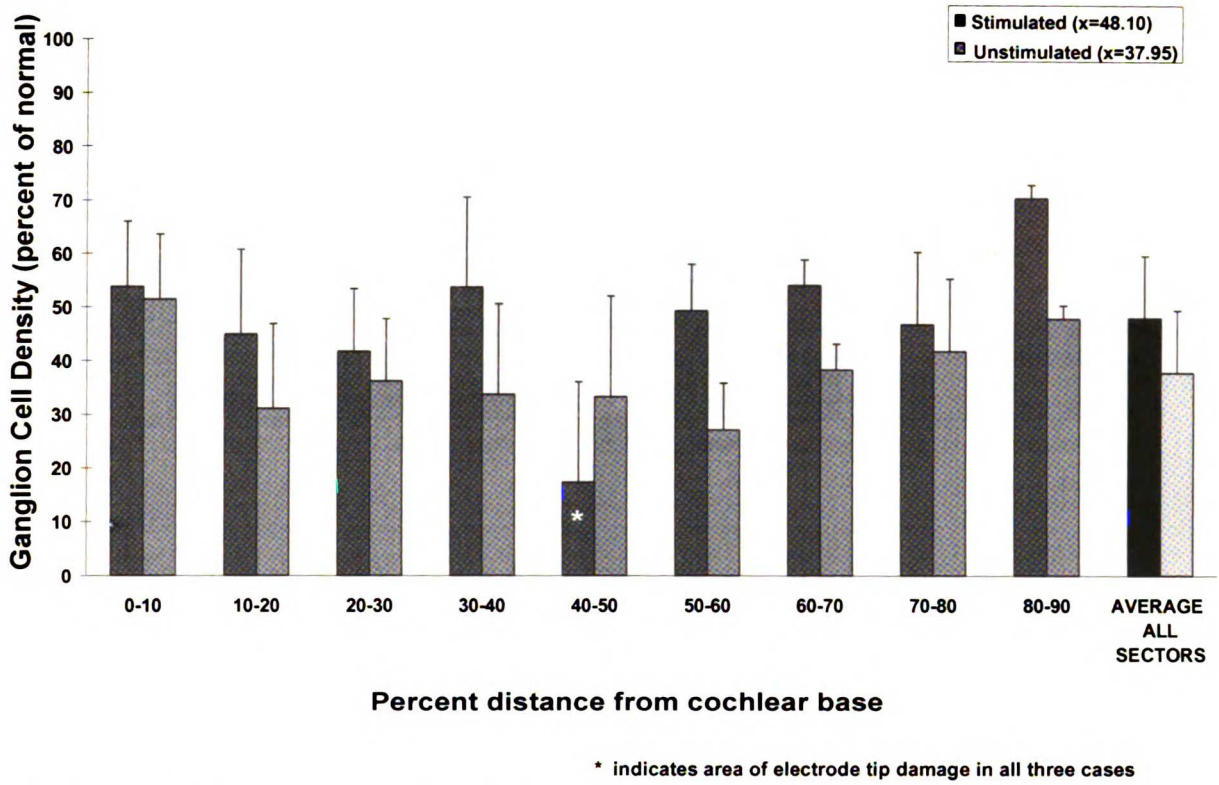


Figure 35. SGC density data for animals completing more than 4 months of chronic electrical stimulation (group 2, n=3). Data are plotted as percent of normal for each 10% sector of the cochlea from base to apex. To the right is the average of all sectors. Error bars indicate standard deviation of the mean.

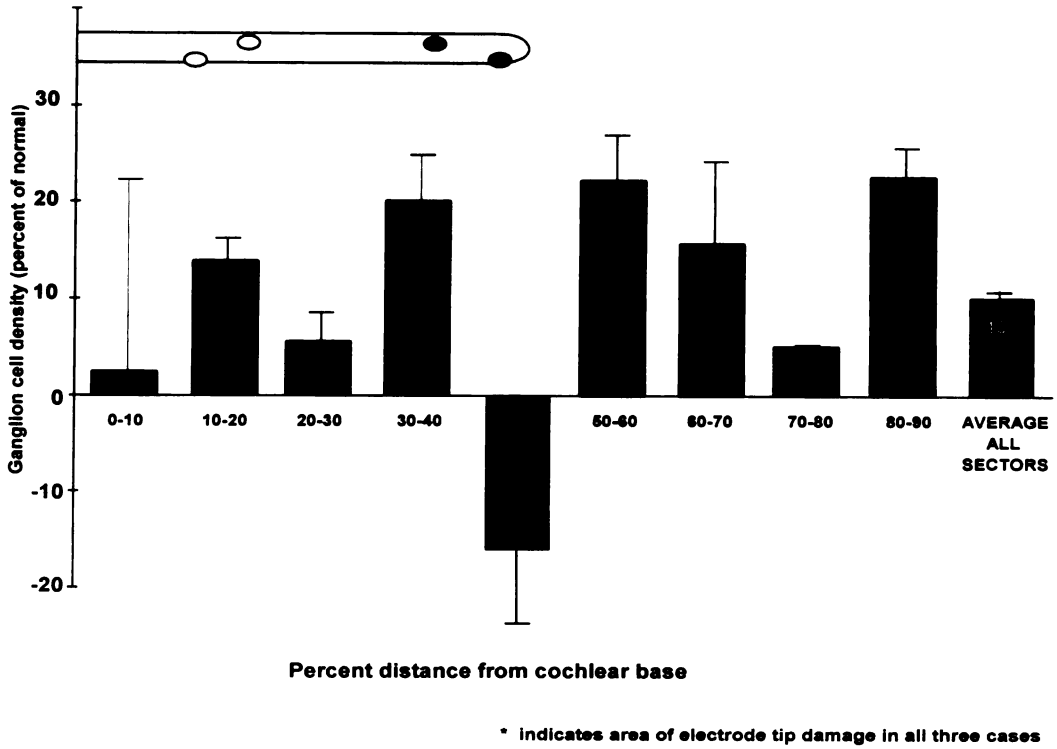
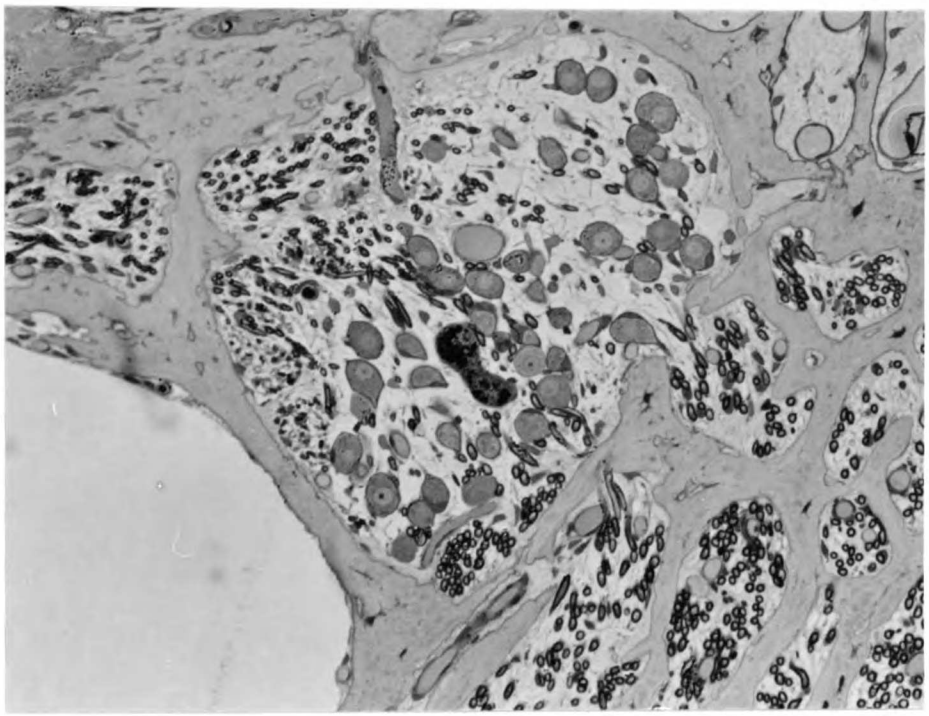


Figure 36. Percent difference in spiral ganglion cell density (stimulated less unstimulated) expressed as percent of normal for the three cats in Group 2. Standard deviations are given for each difference bar. Electrode with stimulating contacts at approximate cochlear location is shown schematically.

a



b

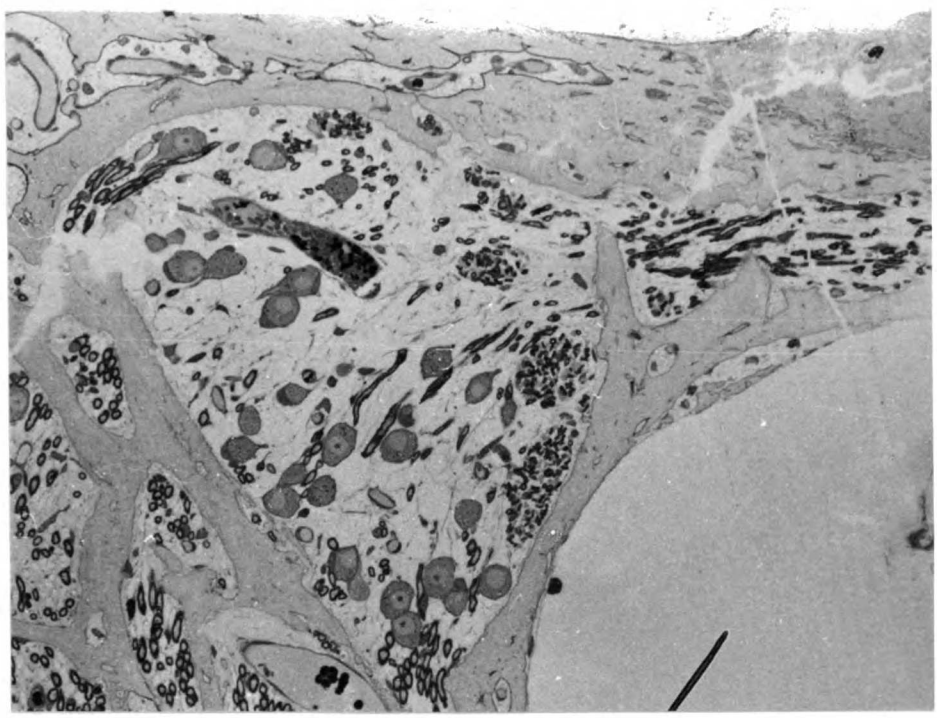


Figure 37ab. Representative photomicrographs showing the spiral ganglion cell survival for the 50-60% region of animal #497. The stimulated ear (**a**) had a 44.6% of normal spiral ganglion cell density, whereas the unstimulated ear (**b**) had a 24.7% survival.

An alternative way to present these data that has been used frequently by other investigators is relative difference. This calculation expresses the percent difference proportionate to the density in the control unstimulated ear. These values are calculated by subtracting the control (unstimulated) value from the experimental (stimulated) value, then dividing by the control value and multiplying by 100 to give percent increase or decrease relative to the control status of the neural population after deafening for a particular animal. **Figure 38** shows the data expressed in this manner. The SGC data again show the greatest increases in survival in the two cochlear sectors immediately adjacent to the stimulating electrodes. Further, a proportionate increase of almost 100% (a doubling of cell density) is seen in the 50-60% region, where SG survival was 27% of normal in the control ears vs. 52% in the stimulated group. However, at the apex (80-90% region) a more modest 40% increase is calculated where survival in the control was 50% of normal vs. 70% in the stimulated ear. Averaged throughout the cochlea, the proportionate increase in SGC density in the stimulated ears was 27% over the paired control cochleae.

3.3.4 Data from Control Deafened Cochleas

The morphometric data from the unstimulated 'control' deafened ears are presented in **Figure 39**. As in all graphs previous to Figure 38, the data here are again plotted as percent of normal for each 10% sector from base to apex. To the right of the graph is the average overall survival. These results suggest that SGC loss was relatively

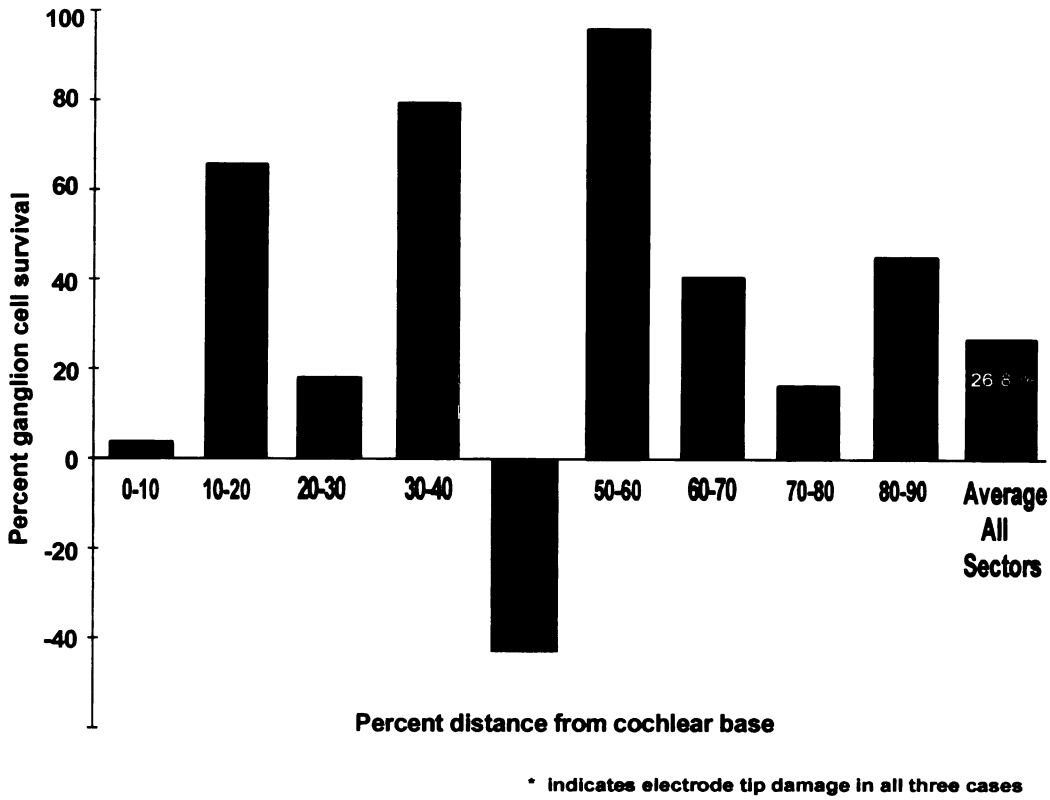


Figure 38. Proportionate difference in SGC density (Stimulated - Unstimulated/Unstimulated x 100) for Group 2 (n=3).

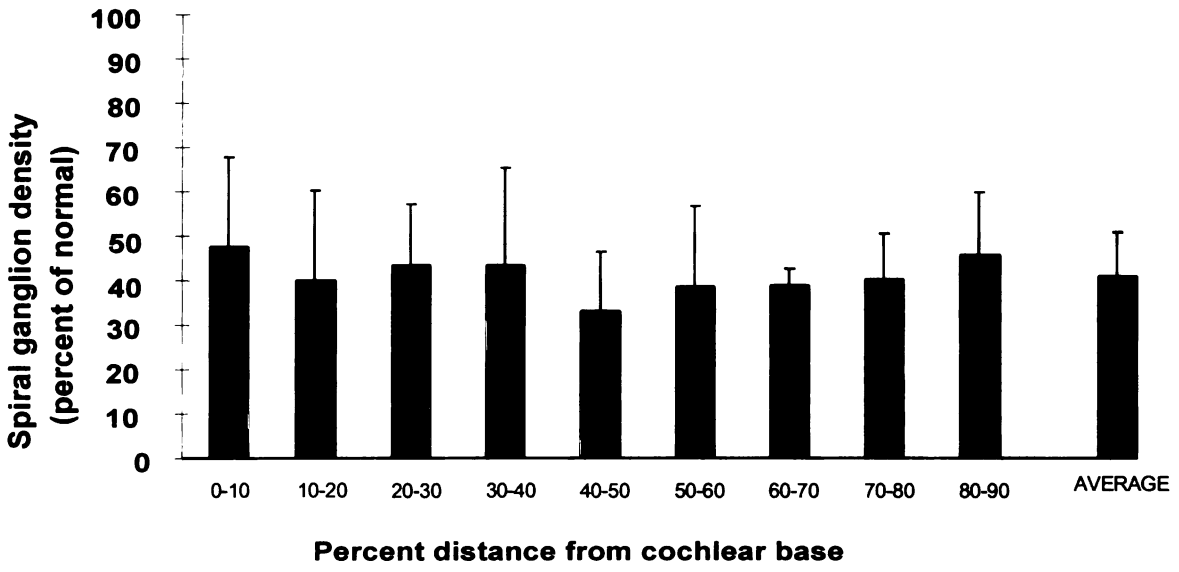


Figure 39. The regional SGC density is shown for the deafened control ears of all animals (Group 1 and Group 2, n=6).

uniform throughout the cochlea and ranged between 30-47%. The mean survival averaged over all areas was 37.5% of normal for this group, which had a mean duration of deafness of 27 weeks.

The graph in **Figure 40** shows SGC density in the control deafened cochleae as a function of duration of deafness for each of the individual adult cats studied (n=6). There is obviously a great deal of scatter in the individual data points, reflecting great individual variability in the ototoxic drug effect. However, the regression function (solid line) suggests that loss of SGC in the adult animals was progressive over time after deafening by co-administration of kanamycin and ethacrynic acid. For comparison, the broken line in **Figure 40** shows a regression function for SG data from the control, unstimulated cochleas of a large group of cats neonatally deafened by administration of neomycin sulfate (Leake et al., unpublished data), age matched to the animals in the present study for duration of deafness. The two functions are fairly similar, suggesting that the time course and extent of cell loss for a given duration of deafness are similar in the 2 groups of animals, although substantial individual variation is noted. Furthermore, the regression line for the adult deafened data is lower suggesting that neural degeneration is perhaps more severe in the adult deafened group for equivalent periods of deafness.

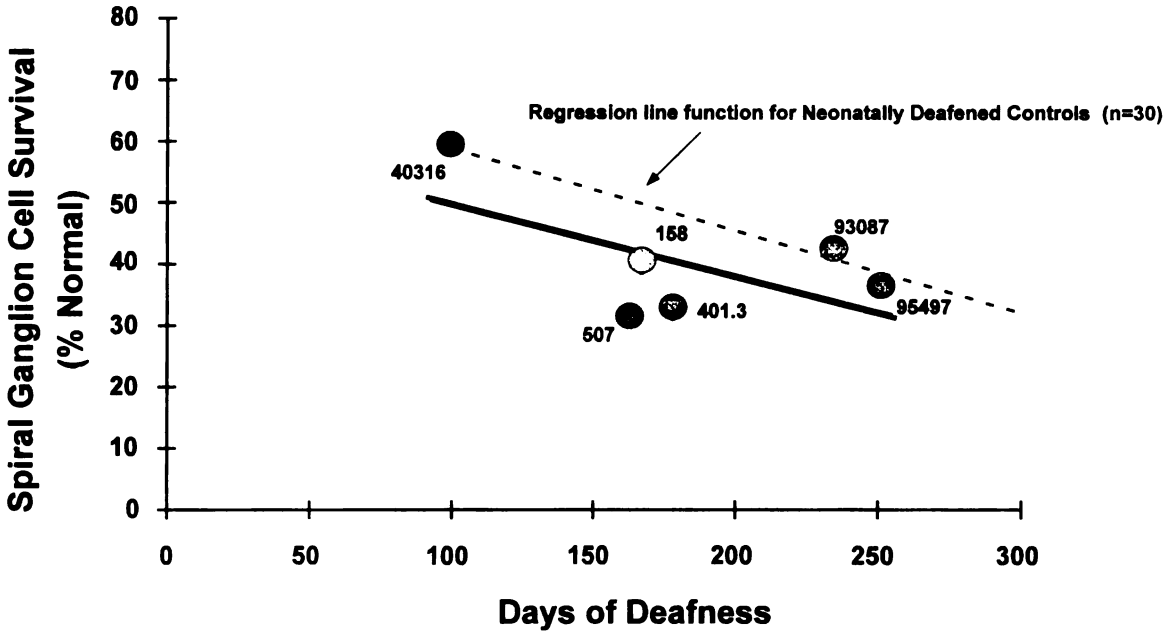


Figure 40. Spiral ganglion cell survival of unstimulated (control) ears versus length of deafness.

4.0 DISCUSSION

The goal of this study was to determine the consequences of chronic electrical stimulation on the peripheral and central auditory system on an animal model of adult onset deafness. It has been shown that chronic intracochlear electrical stimulation in animals deafened neonatally produces alterations in the physiological response properties of neurons in the central auditory system and promotes cell survival at least in the peripheral auditory system. Since these effects have been observed primarily in cats deafened at *birth*, the effects on a mature mammalian auditory system and the potential influence of critical periods of development on the results is unknown. Thus, it is important to examine the effects of chronic intracochlear electrical stimulation on the auditory system that has undergone normal maturation. Understanding the fundamental effects of chronic electrical stimulation on the auditory system in an animal model of adult deafness is an important step in understanding differences in performance among human cochlear implant users and may contribute to improved design and implementation of cochlear implants.

This study proposed to answer the following questions in the adult deafened cat: **1)** What are the characteristic response properties of IC neurons following many months of patterned, chronic intracochlear electrical stimulation? **2)** Does chronic intracochlear electrical stimulation of a relatively restricted cochlear sector alter spatial selectivity (frequency representation) in the inferior colliculus? **3)** If the temporal resolution and/or the spatial selectivity of IC neurons are altered, is it possible to monitor changes longitudinally with a far-field, non-invasive recording

technique, such as the electrically evoked auditory brainstem response (EABR)? **4)** Does chronic electrical stimulation promote spiral ganglion cell (SGC) survival in the mature animal?

In order to address these questions the adult deafened cat was selected for study. Much of the available data on the encoding of acoustic signals and the effects of electrical stimulation on the peripheral and central auditory system have been documented in this animal. Ototoxic drugs were administered to produce a complete, profound sensorineural hearing loss, thereby diminishing the likelihood of remaining hair cells. Animals were chronically stimulated with a 300pps/AM at 30 Hz signal which has been shown to alter temporal and spatial resolution in neonatally deafened cats (Snyder et al., 1995). The auditory midbrain (inferior colliculus) was selected for physiological study since its cochleotopic organization has been described in detail in response to both acoustical and electrical stimulation (Merzenich and Reid, 1974; Black et al., 1983; Snyder et al, 1990). This precise cochleotopic organization of the IC allows the inference of relative frequency and selectivity of electrical stimuli in deaf animals. Further, the effects of chronic intracochlear electrical stimulation have been studied and described for the IC of the neonatally deafened cat (Snyder et al, 1995; Vollmer et al, 1998; In press) and the trophic effects of deafness and chronic stimulation on spiral ganglion cell survival have been examined extensively for the ototoxically deafened kitten (Leake, et al, 1991; 1992; 1995; 1997).

4.1 Deafening

As reported by Xu et al. (1993) administration of kanamycin followed by ethacrynic acid (EA) infusion resulted in a rapid, permanent bilateral hearing loss in adult cats. In the present study, hearing losses were confirmed by the absence of auditory brainstem responses during drug infusion and again just prior to cochlear implantation (see **Figure 4**). In addition, final cochlear morphological study confirmed a complete loss of inner and outer hair cells (see **figure 28**). Moreover, spiral ganglion cell loss in the control ears appeared to be fairly uniform throughout the cochlea with perhaps slightly less loss in the extreme base.

Gradual infusion of the ethacrynic acid allowed dosages to be titrated to individual animals using only the amount necessary to induce profound deafness. This is important since excessive amounts of this drug are nephrotoxic and may jeopardize the general health of the animal. Although two of eleven animals displayed some initial recovery of hearing (within 15 minutes after cessation of EA infusion) additional administration of ethacrynic acid resulted in profound sensory hearing losses. The finding that some hearing initially recovered suggests that continued monitoring of the ABR may be necessary to ensure profound sensorineural hearing loss. Since permanent profound hearing loss was a requirement for this study drugs were administered until no recovery of hearing was observed for at least two hours. Although not determined here, it is possible that even without additional drug administration a profound hearing loss may have ultimately occurred in the animals whose hearing recovered. This should be a consideration for future study.

Blood, urea, nitrogen (BUN) and creatinin levels were within normal limits for all animals immediately post deafening. Kidney dysfunction was confirmed one month later in the one animal that received more than 25 mg/kg of EA (See section 3.1; Table 2, animal # 114). These findings are consistent with the report of Xu et al. (1993), who noted no increase in blood serum concentrations of urea and creatinine levels in their animals immediately after deafening, as long as EA dosage did not exceed 25 mg/kg. For future studies it is recommended that the BUN and creatinine levels be monitored at regular intervals post deafening, since compromised kidney function may occur with longer survival times, especially if a high dose of EA is administered.

4.2 Electrically evoked auditory brainstem response (EABR)

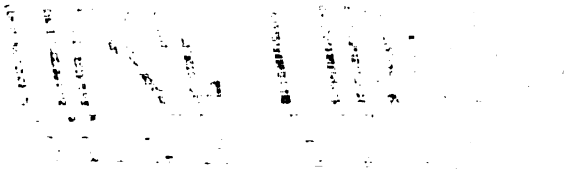
The auditory brainstem response (ABR) to *acoustic* signals has been used as a screening tool for assessment of hearing loss in human subjects unable to provide reliable behavioral responses. Studies have suggested that ABR thresholds are at least within 10 dB of behavioral or psychophysical thresholds (Gorga et al., 1978; Jerger and Mauldin, 1978) and that the two measures may even be within a few dB of one another (Pratt and Sohmer, 1978). The ABR to *electrical* pulses also has been used as a predictor of hearing thresholds for cochlear implant patients who are unable to provide reliable behavioral responses and also for experimental animals. A comparison of physiological and behavioral responses to electrical stimuli has suggested fairly close agreement between these measures, with ABR thresholds to

electrical stimuli approximately 4 dB higher than psychophysical thresholds in cats (Vollmer et al., 1999; Beitel et al., In press).

Additionally, EABR thresholds have been closely correlated to central auditory system single neuron thresholds for the cat (Snyder et al., 1995; Beitel et al., In press). However, comparisons of response thresholds from cochlear nucleus neurons and auditory nerve fibers to electrical signals suggests that the physiological thresholds are slightly higher than behavioral thresholds (Suga and Hattler, 1970; Pfingst, 1988).

Since close agreement between EABR and behavioral and single unit thresholds to electrical signals has been suggested, the EABR was utilized in this study as a fast and noninvasive and reliable means of quantifying threshold. EABR thresholds for many electrode combinations could be obtained in less than 15 minutes in a sedated animal. Thus, the intensity levels for daily chronic electrical stimulation could be set at a constant level of 2 dB above the EABR threshold immediately after implantation. Further, the longitudinal recording of EABRs permitted monitoring device function and adjusting the level of stimulation as necessary to maintain an intensity of 2 dB above threshold.

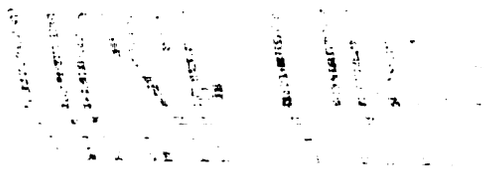
During the course of this study the EABR threshold for stimulating electrode pair 1,2 became elevated in all animals. Despite periodic EABR measurements, constant stimulation at 2 dB above threshold may have been difficult to maintain depending on the time between EABR measures and threshold changes. However, since the EABR in cat has been reported to be an overestimation of perceptual thresholds as estimated by behavioral training, and no animal in this study displayed



more than a 6 dB increase in threshold between recording sessions, it was assumed that all animals received audible stimulation throughout their chronic stimulation period. Furthermore, it was unlikely that the EABR greatly underestimated threshold since no animal reacted adversely or displayed signs of discomfort to daily stimulation.

The increased threshold of pair 1,2 over the course of several months is presumed to reflect a decrease in the local neural population. Such a regional cell loss can result in an increase in the current necessary to stimulate sufficient populations of neurons for generation of an evoked response. In fact, final morphological data from all chronically stimulated animals showed that SGC survival was substantially lower in the region nearest the stimulating electrodes (40-50% cochlear sector) than in any other sector of the cochlea. This loss of SGCs has been attributed to the electrode insertion trauma (see **Figure 34**). It seems likely that this damage and subsequent loss of SGC in this area were at least partially responsible for the gradually increasing EABR thresholds on the apical stimulating pair (1,2) in these animals.

EABRs recorded immediately after cochlear implantation revealed threshold differences between the two stimulating electrode pairs (1,2 and 3,4). Initial mean response threshold for electrode pair 1,2 was 84 μAmps, whereas pair 3,4 required more than twice that level (204 μAmps) to generate a response. In the majority of animals pair 3,4 thresholds decreased (improved) over time, whereas pair 1,2 thresholds elevated (increased) in all animals. Sources of these threshold differences and subsequent changes in physiological thresholds were not the objective of this study, but are important for the interpretation of the results. One hypothesis for the



initial difference between pair 1,2 and pair 3,4 is that the initial loss of perilymphatic fluid which can occur with the surgical fenestration of the round window and insertion of the implant, may result in an elevation in threshold due to the inefficient conductance of current through the altered scala tympani. Electrode pair 1,2 would less likely be effected by a loss of perilymph since it resides deeper within the cochlea and the electrode tip usually is in close contact with the basilar partition as evidenced by the insertion trauma. Further, the anatomical geometry of the cat cochlea places electrode pair 1,2 closer to the SGCs and auditory nerve fibers than pair 3,4 and accordingly may require less current for excitation. (The cat scala tympani markedly tapers in dimension from base to apex (Igarashi, 1976)).

Past studies have suggested that cochlear ossification and tissue growth could lead to increased thresholds (Geier et al., 1993; Balkany et al., 1988). However, in this study the two animals with significant damage in the basal region of the cochlea (# 507, #158) did not exhibit significant increases in EABR thresholds for the basal pair (3,4). Despite the relatively large amount of histologically identified reactive fibrotic connective tissue and bone in the basal region of the cochlea, the EABR thresholds for these animals remained relatively stable. Thus, perhaps such tissue growth results in threshold elevation only when it displaces the electrode farther away from the SGC. In the present study it seems likely that growth of connective tissue around the electrode occluded the fluid space between the electrode and the basilar partition, thus reducing fluid shunting of current and reducing thresholds.

Comparison of EABR thresholds and physiological thresholds recorded within the IC found a fairly good positive correlation (r=0.6) (see **Figure 24**). This finding

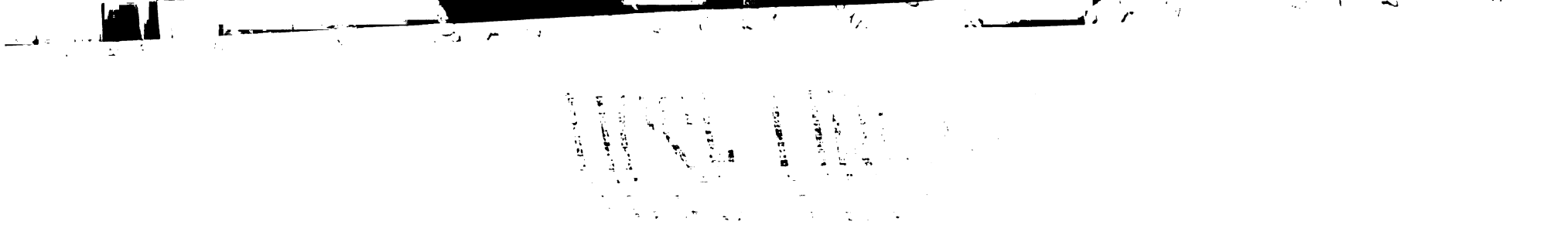
suggests that the EABR threshold provides a reliable measure for assessing device function and setting current levels for chronic stimulation.

Although very little is known about the spread of excitation within the cochlea for electrical stimuli, the STC data (see e.g. **Figure 21**) suggest that the levels of chronic electrical stimulation were sufficient to activate a fairly broad range of frequencies within the central auditory system. Thus, despite substantial regional variations in neural degeneration seen along the cochlear spiral within and between animals, the EABR threshold provided a reliable measure of the current level required for activation of the central auditory system.

4.3 Interpulse Interval Series (IPI)

This study examined the differences in EABR amplitude with varied stimulus pulse rates before and after several months of chronic electrical stimulation. It was hypothesized that changes in temporal resolving capabilities induced by chronic electrical stimulation could be observed as changes in the adaptation of the EABR to increasing rates of electrical stimulation. Current cochlear implant processors are being designed to incorporate higher rates of stimulation in an attempt to adequately represent rapidly changing acoustic signals. Therefore, examination of response properties of the central auditory system to high stimulation rates is an important step in understanding performance differences among cochlear implant users and processing strategies.

The electrically evoked auditory brainstem response (EABR) to increasing rates of pulsatile stimulation was examined in all animals after implantation and



following several months of chronic electrical stimulation. It is known that the EABR amplitude decreases with increasing rates of stimulation and it was hypothesized that changes in the temporal following capacity of the central nervous system would be reflected in the adaptation function of the far-field recorded EABR. A single 'rate' value was obtained by calculating the rate (stimulus frequency) at which the EABR wave III amplitude was reduced by one half. Obtaining a single value permitted comparison of values within and between animals before and after chronic stimulation and to single unit IC data (Fmax).

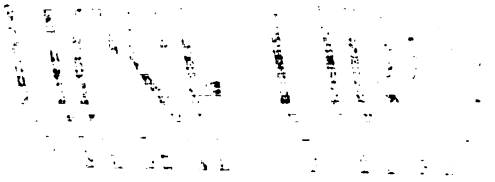
Although the number of animals completing the study was too small for statistical analysis of the data, the data suggested that changes in temporal resolving capabilities may in fact be revealed by this far-field recording method. Three of four animals displayed an increase in frequency following, measured as described above, after several months of chronic electrical stimulation. However, all of the animals showed an increase in temporal resolution as measured by mean Fmax of isolated neurons in the IC.

For this study the IPI series EABRs were recorded at 6 dB above threshold. The 6dB value was originally selected to permit comparison to the pulse frequency following series typically recorded at 6 dB above threshold in the acute physiological studies. Additionally, studies have shown that auditory nerve fibers reach saturation at about 6 dB above threshold for electrical stimuli (Hartman et al., 1984). However, because EABR thresholds varied over time within and between animals, recordings at 6 dB above threshold were made at quite widely varied current levels (μAmp) above threshold. That is, whereas the 6dB increment was held constant for all animals, the

absolute increase in current level (μAmp) differed significantly. For example, a 6 dB increase above a 50 dB threshold is a 185 μAmp increase in current. However, an increase of 6 dB from 60 dB threshold is only a 58 μAmp increase. In future studies, it may provide a better control of stimulation intensity to use a specific current level (e.g. 30 μA) above threshold. This would maintain an equivalent increase in current for each animal and within an animal between recording sessions.

Similarly, the recording of the IPI series at 6 dB above threshold may be affected by an individual animal's growth of magnitude and dynamic range. Although pilot data suggested that there were no systematic differences in the frequency at which the EABR decreased in amplitude by half (half-amplitude) measured at 4, 6 and 8 dB above threshold, the possibility of varied growth functions and dynamic ranges for individual animals has not been addressed in this study and should be examined in future studies.

In retrospect it may have been beneficial also to evaluate the IPI responses of the unstimulated electrode pair 3,4. Since this electrode pair had essentially no shift in threshold over time therefore, EABRs would have been measured at comparable intensity levels before and after chronic stimulation. In contrast, since the threshold for pair 1,2 increased over time, pre and post-chronic stimulation EABRs were measured at different intensity levels. Final IPI series EABRs required higher intensity levels, perhaps resulting in stimulation of a different region of the cochlea and elicitation of responses from different neural elements, thereby producing varied EABR responses. As mentioned previously the SGC degeneration that occurred over time, especially at the electrode tip location (1,2), presumably contributed to the

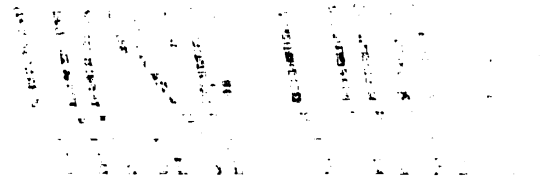


increased neural threshold. This fact also supports the notion that the increased current level required for stimulation is likely to stimulate other/additional regions of the cochlea causing changes in the spatial and temporal summation of inputs.

In summary, these evoked potential data should be taken as a preliminary investigation given the small sample size and limited number of measures. Goals for future studies include a close examination of the effects of intensity and pulse duration on the adaptation function of the EABR. These are important signal parameters that must be examined since current cochlear implant processors utilize not only faster repetition rates but also shorter phase duration signals; therefore, examination of the responses to varied signals is important to consider.

EABR Amplitude Changes: Mechanisms

The finding of a larger mean amplitude of the EABR response after chronic stimulation suggests that a larger number of neurons are responding in a synchronized manner to the stimulus. The potential mechanisms contributing to changes over time in the EABR to varied rates of stimulation are not known, but they presumably reflect some type of increased excitability in the central auditory system after chronic stimulation. It seems likely that the highly synchronized input from the chronic electrical stimulation might powerfully reinforce already existing, excitatory synaptic pathways entraining them to respond. In addition to reinforcement of excitatory synaptic activity there could be long term changes in ascending, descending and/or local inhibitory circuits. Reduction of inhibition would result in greater temporal



summation of central auditory neurons and thus result is an increase in the amplitude of the EABR.

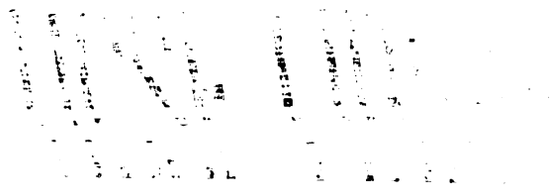
Further support for this inhibitory hypothesis has been suggested by studies examining ABR and postauricular myogenic response amplitudes of animals with selective high frequency hearing losses and ipsilateral cochlear destruction (Harrison et al., 1993; Popelár et al., 1994). Additionally, in similar experimental preparations, enhancement in the evoked potential amplitudes recorded from the IC has been observed (Salvi et al., 1990; Szczepaniak and Møller, 1996). These data have suggested that the mechanisms behind increased responsiveness may be related to decreased inhibition.

4.4 Temporal resolution of IC neurons

4.4.1 Maximum frequency following

One major goal of this study was to examine the effects of chronic electrical stimulation on the temporal resolving capacity of IC neurons in *adult* deafened animals. Past studies have shown that neonatally deafened chronically stimulated animals demonstrate increased temporal resolution following chronic stimulation (Snyder et al., 1995; Vollmer et al., In press). However, the role of development in these changes is unknown. Therefore, this study was designed to determine whether or not similar plasticity can be induced in the *mature* auditory system.

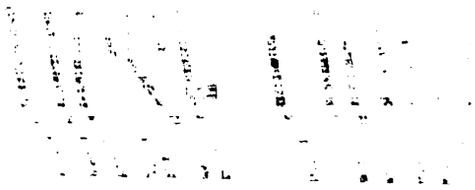
Past studies have shown that adult deafened control (unstimulated) animals have an average Fmax of less than 100 pps (range 10-340 pps) (Snyder et al., 1995) and chronically stimulated neonatally deafened animals have an Fmax of approximately



138-140 pps (Snyder et al., 1995; Vollmer et al., In press). In addition, recent data suggest that the parameters of stimulation (e.g. frequency; bipolar vs monopolar) differentially affect the temporal response properties of IC neurons (Vollmer et al., In press) as well as the spiral ganglion cell survival (Leake et al., 1995; 1997; In Press). Vollmer et al. found that the frequency of the chronic stimulation signal affected the frequency following capability of IC neurons, such that animals stimulated with 'low' frequency (e.g. 30 pps) unmodulated signals had average frequency following similar to normals, but animals stimulated with 'higher' frequency modulated stimuli had significantly increased frequency following.

The results presented here strongly suggest that chronic ICES of the *adult* deafened auditory system alters the temporal response properties of central nucleus IC neurons. Further, the results suggest that these changes are similar to those reported for neonatally deafened animals chronically stimulated with an identical signal. This study found that chronic electrical stimulation in adult deafened animals with a temporally challenging signal (300 pps, amplitude modulated at 30 Hz) for a period of at least 5 months results in an increase in mean frequency following (137 pps) of ICC neurons as compared to normals (97 pps). Further, the data also demonstrated that in these adult animals there was a significant difference in the frequency following capacity of neurons dependent upon their anatomical location. The average Fmax of neurons in the ICE (54 pps) was significantly lower than that of neurons located in the ICC (142 pps).

Although as mentioned previously these chronically stimulated adult animals showed a significant increase in frequency following in the ICC as compared to



normals, it should be noted that no difference in the response properties of ICE neurons was found.

It might be noted that Vollmer et al. (In press) reported that neonatally deafened animals stimulated with temporally challenging stimuli (like those used in the present study) acutally had slightly higher frequency following in the ICE as compared to adult deafened (unstimulated) control animals. Although this difference did not reach significance, this may have been because their sample size for ICE neurons was quite small. If the difference between the ICE of neonatally deafened and adult deafened groups persists with a larger sample size in future studies, this would suggest that the ICE is less adaptable to stimulation-induced alterations in the mature animal.

4.4.2 First Spike Response Latencies

The first spike response latencies of ICE and ICC neurons to electrical stimulation were similar in mean and distribution to the latency data reported in other studies of electrical stimulation (Snyder et al., 1991; Vollmer et al., In press). There was a significant difference between the median response latencies of neurons located in the ICE and ICC. Neurons located in the external nuclei had longer median response latencies as well as a larger range of latencies (x=8.78; 4.69-21.42); whereas ICC neurons had overall shorter latencies (x=5.85; 4.52-8.91).

These findings are in agreement with previously reported data obtained in neonatally deafened chronically stimulated animals (Vollmer et al, In press).

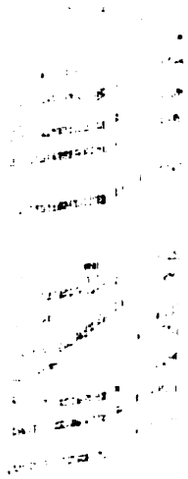
First spike latency differences between the ICE and ICC are likely due to the varied and multiple anatomical pathways that innervate these subnuclei. In addition to

auditory input, the external nucleus receives substantial somatosensory input from distant areas such as the cerebellum and pons (Aitkin 1986). The ICC on the other hand, receives the majority of its inputs directly from the cochlear nucleus and lateral lemniscus (Aitkin, 1986; Kaas, 1996).

Temporal response properties: Mechanisms for alterations

The specific mechanisms responsible for these changes in temporal resolution (Fmax and latency) of ICC neurons are unknown. However, at the first level of analysis an increase in the temporal resolution must involve an increase in the discharge rate of neurons. Increased discharge rate also has been reported in studies of responses to acoustic signals following cochlear damage induced by noise exposure (Willott and Lu, 1982; Lonsbury-Martin and Martin, 1981). Willott and Lu found that approximately 30% of IC neurons increased their firing rates following noise induced hearing loss. Neurons that originally exhibited only onset responses had sustained excitation after noise exposure. Lonsbury-Martin and Martin reported an increase in the suprathreshold firing rates following short duration moderate-intensity sound exposure for neurons in the IC and cochlear nucleus. It has been speculated that these changes are due to alterations in the balance between the excitatory and inhibitory inputs (Salvi et al., 1978; Willot and Lu, 1982).

Taking advantage of the fact that known neurotransmitters control the excitation and suppression of responses in the IC, studies have directly examined the balance between excitation and inhibition. GABA has been identified as a major inhibitory neurotransmitter in the ICC (Roberts and Ribak, 1987), and when it is blocked by the

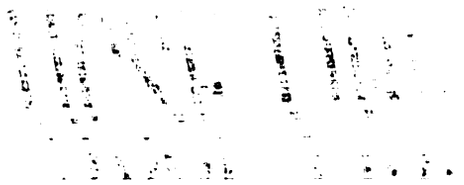


application of bicuculline the firing rate of IC neurons increases (Faingold et al., 1989; Yang et al., 1992). These findings, suggest that inhibitory mechanisms maintain a controlled or balanced response for IC neurons. Removal of this 'balance' changes the way the neuron responds, and in this case it is observed as an increase in firing rate.

Consistent with this theory, the increased firing rate observed in this study could be due to alterations in inhibitory input. Alterations in the response properties of IC neurons created by unilateral chronic stimulation may indicate a decrease or loss of inhibition that results in an increase in maximum firing rate.

Additional mechanisms that could contribute to the plasticity observed in this study include the effects of long-term potentiation (LTP) and mechanisms such as described by Hebb (1949). LTP refers to the long lasting functional changes observed after increased synaptic activity. High frequency synaptic bursts result in increased amplitude of the synaptic response that can be maintained for days or weeks. The actual mechanisms behind LTP have yet to be completely identified, although synaptogenesis and structural alterations have been implicated (Lee et al., 1980; Woolley et al., 1990). Alternatively, Hebb theorized that plasticity results from a strengthening of already existing synaptic connections with constant stimulation. The Hebbian theory would suggest that synchronous electrical stimulation used in this study would result in a strengthening of already present, but relatively weak neuronal connections.

Either of these mechanisms, LTP or Hebbian mechanisms, could be responsible for the increased maximum firing rate observed in these chronically stimulated adult deafened animals. The synchronized activity in auditory neurons induced by the

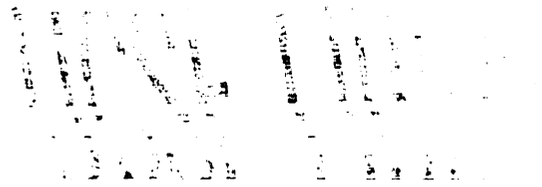


chronic electrical stimulation used in this study could strengthen already existing neuronal connections and/or create additional connections, thereby increasing the firing ability of neurons.

4.5 Spatial tuning curves

Threshold versus depth functions (spatial tuning curves), displayed 'W' shaped curves in which two best locations (minima) usually were evident, one in the external nucleus and the other at depths corresponding to the central IC. The high threshold region between these minima delineated the physiological border between the two subnuclei. The depth of the second tip minimum or best location of the curve varied depending on the cochlear position of the stimulating electrode pair (1,2 or 3,4) and corresponded to the known cochleotopic organization of the ICC. The offset of response minima and the non-overlapping parts of the curves observed in STCs of electrode pairs (1,2 and 3,4) suggested excitation of relatively distinct regions of the IC for the different stimulating pairs.

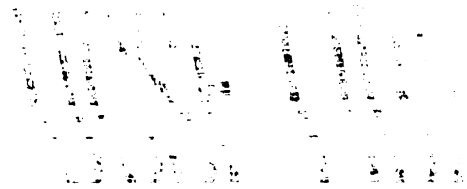
Further, with electrical stimulation of a particular electrode pair (specific cochlear location) at 2 dB above EABR threshold, a relatively restricted area of the ICC was excited. The area of excitation expands as the intensity increases. The central representation of the chronically stimulated electrodes was estimated by measuring the STC width at 6 dB above the minimum threshold for a given penetration (Snyder et al., 1990). The spatial tuning curve widths obtained in these adult animals for the chronically stimulated electrode pair 1,2 was 1.1 mm. This 6 dB width was approximately one and a half times the width reported for normal animals (.74 mm)



(Snyder et al., 1990). These results suggest that chronic electrical stimulation of a single channel of a cochlear implant in animals deafened as adults, after a lifetime of normal auditory experience, resulted in an expansion of the central auditory representation in the ICC as compared to normal animals. On the other hand, the mean STC width observed in these adult deafened cats was not as wide as that reported for neonatally deafened stimulated animals (1.51 mm) (Snyder et al., 1990). Thus, the extent of this spatial expansion induced in adults does not appear to be as great as that observed for neonatally deafened chronically stimulated animals.

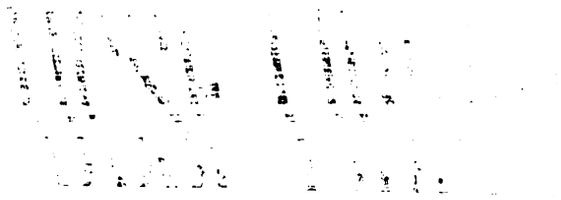
Methodological differences between this study and the Snyder et al. (1990) study could be at least partially responsible for the differences in STC width between adult and neonatally deafened chronically stimulated animals. These differences include the intensity, length and type of chronic stimulation. Snyder et al. stimulated their animals at intensity levels of 6 dB above EABR threshold, whereas, the present study used a more conservative level of 2 dB above threshold. The high levels of chronic stimulation used by Snyder et al. likely stimulated a broader distribution of spiral ganglion neurons (presumably representing a greater range of frequencies) which in turn excited a greater area of the IC, thereby inducing greater expansion and broader mean STC widths.

In the current study no significant differences were measured between the chronically stimulated electrode pair (1,2) and the unstimulated pair (3,4). Although the chronically stimulated electrode pair always had a STC width that was greater than the basal unstimulated pair, this difference was not statistically significant. Snyder et al. reported a significant difference between stimulated and unstimulated electrode



pairs for neonatally deafened animals. Again, these differences may relate to the higher stimulus intensity used in their study and the consequent broader excitation. Moreover, the two studies used markedly different signals for chronic stimulation which may have effected the results. Vollmer et al. (In press) have shown differential effects on the response properties of IC neurons for temporally challenging signals such as the one used in the present study as compared to results obtained when a simple 30 pps signal was used for chronic stimulation in the Snyder (1990) study.

The variations noted between the STCs widths measured in this study also could be due to differences in electrode tip placement (i.e. proximity to SGCs), cochlear pathology (e.g. bone growth vs fibrous tissue) or other factors effecting current spread and excitation patterns. Finally, variations in STC morphology and widths within an animal, such as that noted for animals #158; figure 22a-c, are likely due to variations in the trajectory of the recording electrode with respect to the tonotopic organization of the IC. Although the actual angle of trajectory was held constant throughout the course of an experiment, differences in electrode tracts with respect to the borders of the IC (medial to lateral/posterior to anterior) could result in deviations in the orderly frequency representation within the trajectory with respect to the 'isofrequency' laminae. Overall, the STCs displayed good resolution of the border between the ICE and ICC and the separation between the apical and basal cochlear stimulating electrode pairs.



STC expansion: Mechanisms

There are at least two possible mechanisms for the expansion of STC widths observed following chronic electrical stimulation of these adult deafened animals. The expansion of the ICC region responsive to the electrically stimulated electrode pairs could reflect the establishment of new connections and /or changes in the effectiveness of already existing connections within the IC. Axonal sprouting such as that observed in young and mature animals in response to abnormal visual input (e.g. Darian-Smith et al., 1994) cannot be ruled out in this study. Sprouting could contribute to a change in the anatomical and functional organization of the auditory midbrain. Such changes would likely be reflected as an increase in spatial tuning curve width.

Secondly, chronic stimulation could induce an increase in the effectiveness of already existing, but relatively weak neural connections. The synchronous electrical stimulation of the central auditory pathways could reinforce weak synaptic inputs to the ICC and result in a spatial expansion of the area responsive to the stimulated sector of the cochlea.

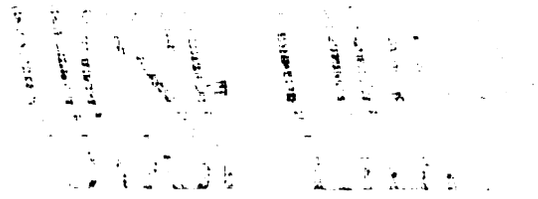
Future studies using neuronal tracers to determine if there are changes in connectional selectivity of projections to the ICC after chronic stimulation would be extremely useful in determining which of these mechanisms underlies the observed functional changes.

4.6 Cochlear Morphology

Past studies have shown that chronic electrical stimulation in neonatally and adult deafened animals can partially prevent the progressive degeneration of spiral ganglion cells that usually occurs following deafening (Lousteau, 1987; Hartshorn et al., 1991; guinea pig; Leake et al., 1991, 1992 and 1995 cat). Although conflicting results have been reported in other studies (Shepherd et al., 1994; Li et al., 1997; Araki et al., 1998), major differences in stimulation paradigms and animal models likely account for these differences. For example, Leake et al. (1991) showed that two months of chronic electrical stimulation was insufficient to demonstrate the trophic effects of chronic stimulation. Thus, one factor which clearly contributes to differences in SGC survival (or the lack thereof) is the duration of the chronic stimulation period.

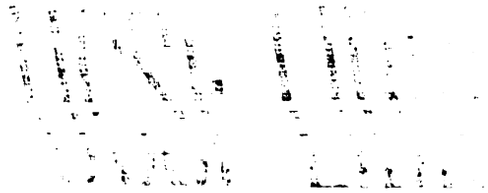
The present study examined the protective effects of temporally challenging electrical stimulation (300 pps/AM at 30 Hz) in an adult model of late onset deafness. Animals with severe, chronic infection and shorter stimulation periods were excluded from the results with respect to the effects of stimulation in maintaining neural survival; however, their control ears provided valuable information.

Although the sample size was small, the data strongly suggest that chronic electrical stimulation in *adult* deafened animals can prevent the degeneration of SGC after deafening. More than 10% of the normal SGC population was maintained in the stimulated cochleae over and above that observed in the deafened unstimulated control ears. Overall, SGC density was maintained at 48% of normal for the stimulated ear, versus 38% of normal in the unstimulated ear. Calculated as percent



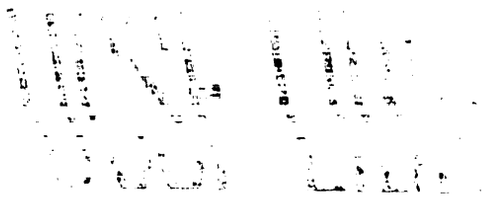
increase over the unstimulated ear, the results demonstrated a proportionate increase of 27% in SGC density in the stimulated ears. This difference for these adult deafened animals was approximately half that reported by Leake et al. (In press) for neonatally deafened animals stimulated with an identical signal and for a similar duration. This finding suggests that electrical stimulation may have a more protective effect on the SGC of young deafened animals, than on the SGC of mature animals. Alternately, the different deafening protocols used in the two studies may have contributed to this difference. In neonatal animals, daily subcutaneous injections of neomycin were administered over a 2-3 week period to induce profound hearing loss. In the present study, the co-administration of kanamycin and ethacrynic acid induced equivalent profound hearing loss in a much shorter time, often in less than an hour. This rapid and severe ototoxic drug effect may induce more severe acute trauma to the organ of Corti. Two findings support this notion of more severe acute ototoxicity. First, for a large portion of the cochlea few supporting cells were present. Secondly, the finding of significant loss of SGC within one of these adult animals (#507) studied only 13 weeks after drug treatment supports the notion of a more severe neurotoxicity induced with the kanamycin and E.A. protocol.

Chronic infection and insertion trauma were obviously detrimental to SGC survival and caused degeneration of neurons beyond that observed simply with ototoxic deafening (see control ear data). Further, it appeared that this damage may prevented the protective effects of chronic electrical stimulation (see **figure 30, animal #507 and 158**). The regional morphometric data showed that the cochlear sector where insertion trauma occurred was the only sector where SGC survival was



poorer in the stimulated ear. This finding clearly demonstrates that damage from traumatic insertion of the implant can offset the trophic effects of stimulation and can result in marked SGC loss in that region.

The importance of the duration of chronic stimulation is evident in the data demonstrating the time necessary to observe progressive degeneration of the SGC. The protective effects of electrical stimulation on SGC survival is dependent upon sufficient degeneration occurring in the contralateral (control) ear to show a compelling difference, despite the individual variability of response to the ototoxic drugs. Examination of the control-deafened ears in these experimental animals suggests a fairly symmetrical loss of ganglion cells throughout the cochlea. Further, the loss appears to progress gradually over time (**see figure 34**). Examination of the control cochlea of one animal deafened less than 3 months shows better survival with approximately 60% SGCs remaining (see **figure 26, animal #403**) as compared to about 37.5% mean survival in the control ears deafened for 7 months. This finding is consistent with the widely accepted notion that once the hair cells are destroyed a secondary degeneration of SGC occurs (e.g. Nadol, 1984; Leake and Hradek, 1988; Leake et al., 1997). Moreover, this requirement for prolonged stimulation periods (>3 months) to demonstrate significant differences between stimulated and control ears, likely explains the failure to see trophic effects in previous studies that used shorter stimulation periods (Shepherd et al., 1994; In press; Araki et al., 1998; Li et al.1997).



Increased SGC survival: Mechanisms

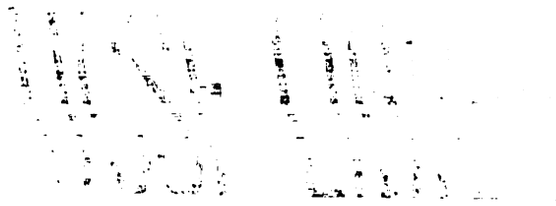
Spiral ganglion cell degeneration is observed secondary to hair cell loss, with retrograde degeneration progressing to the auditory nerve, and transneuronal effects occurring in the brainstem and even within the auditory midbrain (Moore et al., 1986; Pasic and Rubel, 1989; Kitzes 1996; Lachica et al., 1996;). Loss of input from the hair cells is presumed to precipitate these degenerative changes.

This study demonstrates that chronic stimulation prevents some of the SGC degeneration typically observed following the loss of hair cells. The mechanisms underlying the greater SGC survival in the stimulated ear can only be speculated upon, but it appears that reestablishment of input to the auditory system via the cochlear implant is somehow trophic to the SGC neurons. It has been suggested that the electrical stimulation replaces the trophic effects of synaptic input from the hair cells by providing at least some of the metabolic activity necessary for cell survival.

One possible mechanism for this trophic effect could be the increased blood flow observed with electrical stimulation (Sillman et al., 1989; Laurikainen et al., 1994). Studies of the guinea pig cochlea have shown that electrical stimulation causes vasodilation in the inner ear as measured by cerebral blood flow study. This increased blood flow during electrical stimulation could assist in providing nutrients necessary for cellular maintenance.

In addition, molecular studies have shown that neurotrophic factors protect spiral ganglion neurons in tissue culture from ototoxic agents (Zheng et al., 1995) and promote survival of SGC after hair cell loss (Miller et al., 1997). Moreover, it has been demonstrated directly that depolarization promotes survival of of SGC in cell

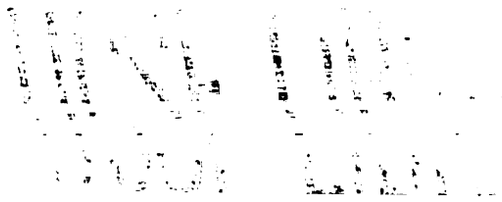
culture (Hegerty et al., 1997). Thus, it is likely that another mechanism underlying neural maintenance induced by electrical stimulation is the upregulation of neurotrophins which promote cell survival.



5.0 CONCLUSIONS: Implications for Cochlear Implant Users

Despite the historic belief that only the young developing nervous system is capable of plasticity, significant alterations have been observed in the mature nervous system. This study provides further evidence that the adult mammalian auditory system is capable of significant plasticity. Specifically, this study demonstrated that chronic electrical stimulation delivered by a cochlear implant can alter the temporal processing and spatial resolution (frequency map) of the central auditory system in the mature cat. Further, this study suggested that plasticity may be demonstrable using the far-field recorded electrically evoked auditory brainstem response. Additionally, the histological results confirmed that electrical stimulation has a trophic effect on SGC survival in this mature animal model.

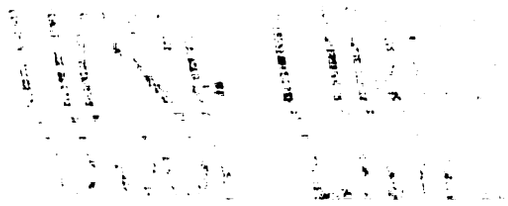
Although these studies were conducted in animals, there are several important implications for human cochlear implant patients. In this study it was demonstrated that chronic electrical stimulation with a temporally challenging signal through a cochlear implant produced significant changes in the temporal and spatial response properties of the central nervous system. It should be noted that this was accomplished with passive stimulation. Even greater change may be induced with attention to the stimulus and learning. For cochlear implant users this finding is important since it suggests that the nervous system is capable of adapting and perhaps modifying to provide the best possible resolution of an 'aberrant' signal. Moreover, it suggests that current cochlear implant users may be able to adapt to and utilize future improvements in signal processing strategies.



One goal of this study was to determine whether or not the EABR to increasing rates of stimulation could be used as a predictor or measure of temporal processing. Temporal processing has been implicated as one variable which may predict an individual's success with a cochlear implant. Therefore, a method to evaluate temporal processing would be of significant benefit to the rehabilitation of cochlear implant patients. Such a measure would be valuable not only for predicting speech performance, but for modifying an individuals' speech processing strategy if expected performance was not met. The ultimate goal of such a measure would be to provide information that could be used to optimally fit an individual with their speech processor. This study provides some evidence that plasticity induced by electrical stimulation may be observed using the easily recorded EABR.

The histological results suggest that for patients presenting with profound sensorineural hearing loss, the adverse effects of auditory deprivation may be at least partially ameliorated with electrical stimulation. Since it has been presumed that spiral ganglion cell survival is one factor effecting results with the cochlear implant, preservation of SGCs should be a consideration when recommending cochlear implantation to a patient.

An additional finding of this study, which has important implications for the design of cochlear implants, is the significant SGC loss that resulted as a consequence of cochlear implant insertion trauma. Significant migration and/or misplacement of the intracochlear array have been observed in high resolution CT scans of human cochlear implant patients (Ketten et al., 1998). In the present study, damage from the electrode tip resulted in severe regional SGC loss. The implications of this damage to



cochlear implant device efficacy was further emphasized by the elevation in EABR threshold recorded with stimulation of the electrode pair (1,2) nearest the damage. This elevation in threshold was presumably due to progressive neural degeneration consequent from the insertion trauma at the electrode tip. This damage offset the trophic effect of electrical stimulation and has important implications for the design of cochlear implants. Clearly, an important priority for future generations of cochlear implants should be the design of electrodes that can be inserted within the scala tympani safely and reliably without trauma.

6.0 BIBLIOGRAPHY

Aitkin, L. (1986). The Auditory Midbrain: Structure and Function in the Central Auditory Pathway. Humana Press: Clifton, New Jersey.

Araki, S., Kawano, A., Seldon, L., Shepherd, R.K., Funasaka, S. and Clark, G.M. (1998) Effects of chronic electrical stimulation on spiral ganglion neuron survival and size in deafened kittens. Laryngoscope, May, 108(5):687-95.

Bakin, J.S. and Weinberger, N.M. (1990) Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. Brain Research, Dec 17, 536(1-2): 271-86.

Bakin, J.S. and Weinberger, N.M. (1996) Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. Proceedings of the National Academy of Sciences of the United States of America, Oct 1, 93(20): 11219-24.

Balkany, T., Gantz, B., Nadol, J.B. Jr. (1988) Multichannel cochlear implants in partially ossified cochleas. Annals of Otology, Rhinology, and Laryngology. Supplement, Sep-Oct, 135:3-7.

Ball, K. and Sekuler, R. (1982) A specific and enduring improvement in visual motion discrimination. Science, Nov 12, 218.

Beitel, R. E., Vollmer, M., Snyder, R.L., Schreiner, C.E., and Leake, P.A. (In press) Behavioral and neurophysiological thresholds to electrical stimulation of the deaf cat cochlea. Audiol. Neuro-Otol.

Black, R.C., Clark, G.M., Tong, Y.C., and Patrick, J.F. (1983) Current distributions in cochlear stimulation. Ann. N.Y. Acad. Sci. 405, 137-145.

Born, D.E. and Rubel, E.W. (1985) Afferent influences on brain stem auditory nuclei of the chicken: neuron number and size following cochlea removal. Journal of Comparative Neurology, Jan 22, 231(4):435-45.

Brown, M., Webster, W.R. and Martin, R.L. (1997) The three-dimensional frequency organization of the inferior colliculus of the cat: a 2-deoxyglucose study. Hearing Research, Feb, 104(1-2):57-72.

Buonomano, D.V. and Merzenich, M.M. (1998) Cortical plasticity: from synapses to maps. Annual Review of Neuroscience, 21:149-86.

Chapman, B. and Stone, L.S. (1996) Turning a blind eye to cortical receptive fields. Neuron, Jan, 16(1):9-12.

Chino, Y.M., Kaas, J.H., Smith, E.L. 3d, Langston, A.L. and Cheng, H. (1992) Rapid reorganization of cortical maps in adult cats following restricted deafferentation in retina. Vision Research, May, 32(5):789-96

Chouard, C.H., Meyer, B., Josset, P. and Buche, J.F. (1983) The effect of chronic electrical stimulation upon the guinea pig cochlear nucleus development. Acta Otolaryngology 95, 639-645.

Crist, R.E., Kapadia, M.K., Westheimer, G. and Gilbert, C.D. (1997) Perceptual learning of spatial localization: specificity for orientation, position, and context. Journal of Neurophysiology, Dec, 78(6):2889-94.

Darian-Smith, C. and Gilbert, C.D. (1994) Axonal sprouting accompanies functional reorganization in adult cat striate cortex. Nature, Apr 21, 368(6473):737-40.

Eggermont, J.J. and Bock, G.R. (1986) Critical periods in auditory development. Acta Otolaryngol. Suppl. 429, 1-64.

Evans, E.F. (1975) Cochlear Nerve and Cochlear Nucleus. In: W.D. Keidel and D.N. Williams (eds), Auditory System. New York: Springer-Verlag

Fahle, M. and Harris, J.P. (1998) The use of different orientation cues in vernier acuity. Perception and Psychophysics, Apr, 60(3):405-26.

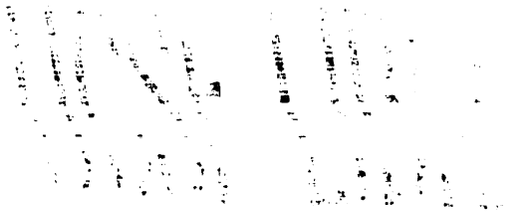
Faingold, C.L., Gehlbach, G. and Caspary, D.M. (1989) On the role of GABA as an inhibitory neurotransmitter in inferior colliculus neurons: iontophoretic studies. Brain Research, Oct 23, 500(1-2):302-12.

Fleckeisen, C.E., Harrison, R.V. and Mount, R.J. (1991) Effects of Total Cochlear Haircell Loss on Integrity of Cochlear Nucleus. Acta Otolaryngol. Suppl. 489: 23-31.

Geier, L., Gilden, J., Luetje, C.M., and Maddox, H.E. 3d. (1993) Delayed perception of cochlear implant stimulation in children with postmeningitic ossified cochleae. American Journal of Otology, Nov, 14(6):556-61

Gerken, G.M.; Saunders, S.S. and Paul, R.E. (1984) Hypersensitivity to electrical stimulation of auditory nuclei follows hearing loss in cats. Hearing Research, 13, 249-259.

Gorga, M.P., Worthington, D.W., Reiland, J.K., Beauchaine, K.A., and Goldgar, D.E. (1985) Some comparisons between auditory brainstem response thresholds, latencies, and the pure-tone audiogram. Ear and Hearing 6, 105-112.



Greenwood, D.D. (1974) Critical bandwidth in man and some other species in relation to the traveling wave envelope. In: H.R. Moskowitz and J.C. Stevens (eds): Sensation and Measurement. Boston: Reidel, pp. 231-239.

Hall, J.W. (1992) Handbook of auditory evoked responses. Boston: Allyn and Bacon.

Harrison, R.V., Stanton, S.G., Ibrahim, D., Nagasawa, A. and Mount, R.J. (1993) Neonatal cochlear hearing loss results in developmental abnormalities of the central auditory pathways. Acta Oto-Laryngologica, May, 113(3):296-302.

Hartmann, R., Topp, G, and Klinke, R. (1984) Discharge patterns of cat primary auditory fibers with electrical stimulation of the cochlea. Hearing Research, Jan, 13(1):47-62.

Hartmann, R., Shepherd, R.K., Heid, S. and Klinke, R. (1997) Response of the primary auditory cortex to electrical stimulation of the auditory nerve in the congenitally deaf white cat. Hearing Research, Oct, 112(1-2):115-33.

Hartshorn, D.O., Miller, J.M. and Altschuler, R.A. (1991) Protective effect of electrical stimulation in the deafened guinea pig cochlea. Otolaryngology and Head and Neck Surgery, Mar, 104(3):311-9.

Hashisaki, G.T. and Rubel, E.W. (1989) Effects of unilateral cochlea removal on anteroventral cochlear nucleus neurons in developing gerbils. Journal of Comparative Neurology, May 22, 283(4):5-73.

Hebb, D.O. (1949) The Organization of Behavior: A Neuropsychological Theory. New York: Wiley.

Hegerty, J.L., Kayl, A.R., and Green, S.H. (1997) Trophic support of cultured spiral ganglion neurons by depolarization exceeds and is additive with that by neurotrophins or cAMP and requires elevation of [Ca2+]i within a set range. Journal of Neuroscience, 6, 1959-1970.

Hultcrantz, M., Snyder, R., Rebscher, S. and Leake, P. (1991) Effects of neonatal deafening and chronic intracochlear electrical stimulation on the cochlear nucleus in cats. Hearing Research, Aug, 54(2):272-80.

Igarashi, M., Takahashi, M., and Alford, B.R. (1976) Cross-sectional area of scala tympani in human and cat. Archives of Otolaryngology, Jul, 102(7):428-9.

Irvine, D.R.F. and R. Rajan (1994) Plasticity of frequency organization in inferior colliculus of adult cats with unilateral restricted cochlear lesions: 17th Midwinter meeting of the Association for Research in Otolaryngology. 82:S21

Jean-Baptiste, M. and Morest, D.K. (1975) Transneural changes of synaptic endings and nuclear chromatin in the trapezoid body following cochlear ablation in cats. J. Comp. Neurol. 162, 111-134.

Jerger, J. and Mauldin, L. (1978) Prediction of sensorineural hearing level from the brain stem evoked response. Archives of Otolaryngology, Aug, 104(8):456-61.

Kaas, J.H. (1991) Plasticity of sensory and motor maps in adult mammals. Annu. Review Neuroscience 14: 137-167.

Kaas, J.H. (1996) Plasticity of Sensory Representations in the Auditory and Other Systems of Adult Mammals. In: R.F. Salvi, D. Henderson, F. Fiorino and V. Colletti (Eds). Auditory System Plasticity and Regeneration. Stuttgart, New York: Thieme.

Kaas, J.H. (1997) Topographic maps are fundamental to sensory processing. Brain Research Bulletin, 44(2):107-12.

Kaltenbach, J.A., Czaja, J., and Kaplan, C.R. (1992) Changes in the tonotopic map of the dorsal cochlear nucleus following induction of cochlear lesions by exposure to intense sound. Hearing Research, May, 59(2):213-23.

Kapadia, M.K., Gilbert, C.D., Westheimer, G. (1994) A quantitative measure for short-term cortical plasticity in human vision. Journal of Neuroscience, Jan, 14(1):451-7.

Ketten, D.R., Skinner, M.W., Wang, G., Vannier, M.W., Gates, G.A., and Neely, J.G. (1998) In vivo measures of cochlear length and insertion depth of nucleus cochlear implant electrode arrays. Annals of Otology, Rhinology, and Laryngology. Supplement, Nov, 175:1-16.

Kilgard, M.P. and Merzenich, M.M. (1998) Cortical map reorganization enabled by nucleus basalis activity. Science, Mar 13, 279(5357):1714-8.

Kitzes, L. (1996) Anatomical and Physiological Changes in the Brainstem Induced by Neonatal Ablation of the Cochlea. In: R.F. Salvi, D. Henderson, F. Fiorino and V. Colletti (Eds). Auditory System Plasticity and Regeneration. Stuttgart, New York: Thieme.

Knudsen, E.I. (1998) Capacity for plasticity in the adult owl auditory system expanded by juvenile experience. Science, Mar 6, 279(5356):1531-3.

Lachica, E.A., Zirpel, L. and Rubel, E.W. (1996) Intracellular Mechanisms Involved in the Afferent Regulation of Neurons in the Avian Cochlear Nucleus in Auditory System Plasticity and Regeneration. . In: R.F. Salvi, D. Henderson, F. Fiorino

Lasky, R.E., Maier, M.M. and Hecox, K. (1996) Auditory evoked brain stem responses to trains of stimuli in human adults. Ear and Hearing, Dec, 17(6):544-551.

Laurikainen, E.A., Costa, O., Miller, J.M., Nuttall, A.L., Ren, T.Y., Masta, R., Quirk, W.S., and Robinson, P.J. (1994) Neuronal regulation of cochlear blood flow in the guinea-pig. Journal of Physiology, Nov 1, 480 (Pt 3):563-73.

Leake, P.A., Snyder, R.L. and Schreiner, C.E. (1987) Cochlear pathology of sensorineural deafness in cats: Coadministration of kanamycin and aminooxyacetic acid. Annals of Otology, Rhinology and Laryngology, 96, 1:48-50.

Leake, P.A. and Hradek, G.T. (1988) Cochlear pathology of long term neomycin induced deafness in cats. Hearing Research, Apr, 33(1):11-33.

Leake, P.A., Hradek, G.T., Rebscher, S.J., and Snyder, R.L. (1991) Chronic intracochlear electrical stimulation induces selective survival of spiral ganglion neurons in neonatally deafened cats. Hearing Research, Aug, 54(2):251-71.

Leake, P.A., Snyder, R.L., Hradek, G.T., and Rebscher, S.J. (1992) Chronic intracochlear electrical stimulation in neonatally deafened cats: effects of intensity and stimulating electrode location. Hearing Research, Dec, 64(1):99-117.

Leake, P.A., Snyder, R.L., Hradek, G.T., and Rebscher, S.J. (1995) Consequences of chronic extracochlear electrical stimulation in neonatally deafened cats. Hearing Research, Jan, 82(1):65-80.

Leake, P.A., Kuntz, A.L., Moore, C.M., and Chambers, P.L. (1997) Cochlear pathology induced by aminoglycoside ototoxicity during postnatal maturation in cats. Hearing Research, Nov, 113(1-2):117-32.

Leake, P.A., (In press) Long term effects of chronic electrical stimulation. In Cochlear Implants. Eds. Waltzmann, S.B., Cohen, N. Theime: New York.

Lee, K.S., Schottler, F., Oliver, M., and Lynch, G. (1980) Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus. Journal of Neurophysiology, Aug, 44(2):247-58.

Li, L., Webster, D.B., and Parkins, C.W. (1997) 20th Midwinter meeting. Association for Research Otolaryngology. 314.

Li, L., Parkins, C.W., and Webster, D.B. (In Press) Hearing Research.

Liberman, M.C. (1982) The cochlear frequency map for the cat: Labeling auditory-nerve fibers of know characteristic frequency. J. Acoustic. Soc. Am. 72: 1441-1449.

Lonsbury-Martin, B.L. and Martin, G.K. (1981) Effects of moderately intense sound on auditory sensitivity in rhesus monkeys: behavioral and neural observations. Journal of Neurophysiology, Sep, 46(3):563-86.

Lousteau, R.J. (1987) Increased spiral ganglion cell survival in electrically stimulated, deafened guinea pig cochleae. Laryngoscope, Jul, 97(7 Pt 1):836-42.

Lustig, L.R., Leake, P.A., Snyder, RL. and Rebscher, S.J. (1994) Changes in the cat cochlear nucleus following neonatal deafening and chronic intracochlear electrical stimulation. Hearing Research, Apr, 74(1-2):29-37.

Mardia, K.V. (1972) Statistics of directional data. London: Academic Press.

Matsushima, J.I., Shepherd, R.K., Seldon, H.L., Xu, S.A., and Clark, G.M. (1991) Electrical stimulation of the auditory nerve in deaf kittens: effects on cochlear nucleus morphology. Hearing Research, Nov, 56(1-2):133-42

McGee, T.M. and Olzewski, J. (1962) Streptomycin suphate and dihydrostreptomycin ototoxicity: behavioral and histopathological studies Arch of Otolaryngology 75: 295-311.

Melzer, P. and Smith, C.B. (1998) Plasticity of cerebral metabolic whisker maps in adult mice after whisker follicle removal--I. Modifications in barrel cortex coincide with reorganization of follicular innervation. Neuroscience, Mar, 83(1):27-41

Merzenich, M.M., Michaelson, R.P., Pettit, C.R., Schindler, R.A. and Reid, M. (1973) Neural encoding of sound sensation evoked by electrical stimulation of the auditory nerve. Ann. Otol. 83, 486-504.

Merzenich, M.M. and Reid, M.D. (1974) Representation of the cochlea within the inferior colliculus of the cat. Brain Research, Sep 13, 77(3):397-415.

Merzenich, M.M., Nelson, R.J., Stryker, M.P., Cynader, M.S., Schoppmann, A. and Zook, J.M. (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. Journal of Comparative Neurology, Apr 20, 224(4):591-605.

Miller, J.M., Chi, D.H., O'Keefe, L.J., Kruska, P., Raphael, Y., and Altschuler, R.A. (1997) Neurotrophins can enhance spiral ganglion cell survival after inner hair cell loss. International Journal of Developmental Neuroscience, Jul, 15(4-5): 631-43.

Moore, D.R. and Irvine, D. (1981) Plasticity of binaural interaction in the inferior colliculus. Brain Research, 208; 198-202.

Moore, D.R. and Kitzes, L.M. (1986) Cochlear nucleus lesions in the adult gerbil: effects on neurone responses in the contralateral inferior colliculus. Brain Research, May 14, 373(1-2):268-74.

Moore, D.R. and Kowalchuk, N.E. (1988) Auditory brainstem of the ferret: effects of unilateral cochlear lesions on cochlear nucleus volume and projections to the inferior colliculus. Journal of Comparative Neurology, Jun 22, 272(4):503-15.

Movshon, J.A. and Van Sluyters, R.C. (1981) Visual neural development. Annual Review of Psychology, 32:477-522.

Murayama, Y., Fujita, I., and Kato, M. (1997) Contrasting forms of synaptic plasticity in monkey inferotemporal and primary visual cortices. Neuroreport, Apr 14, 8(6):1503-8.

Nadol, J.B. Jr. (1984) Histological considerations in implant patients. Archives of Otolaryngology, Mar, 110(3):160-3.

National Institutes of Health (NIH). Consensus Development Conference on Cochlear Implants in Adults and Children. May 15-17, 1995.

Nudo, R.J., Jenkins, W.M. and Merzenich, M.M. (1990) Repetitive microstimulation alters the cortical representation of movements in adult rats. Somatosensory and Motor Research, 7(4):463-83.

Ojemann, G.A. (1994) Inside the brain: mapping the cortex, exploring the neuron. W. H. Calvin, and G.A. Ojemann (eds). New York : New American Library.

Osberger, M.J., Todd, S.L., Berry, S.W., Robbins, A.M., and Miyamoto, R.T. (1991) Effect of age at onset of deafness on children's speech perception abilities with a cochlear implant. Ann. Otol. Rhinol. Laryngology; 100: 883-888.

Pasic, T.R. and Rubel, E.W. (1989) Rapid changes in cochlear nucleus cell size following blockade of auditory nerve electrical activity in gerbils. Journal of Comparative Neurology, May 22, 283(4):474-80.

Pfingst, B.E. (1988) Comparisons of psychophysical and neurophysiological studies of cochlear implants. Hearing Research, Aug, 34(3):243-51.

Popelár, J., Erre, J.P., Aran, J.M. and Cazals, Y. (1994) Plastic changes in ipsi-contralateral differences of auditory cortex and inferior colliculus evoked potentials after injury to one ear in the adult guinea pig. Hearing Research, Jan, 72(1-2):125-34.

Pratt, H and Sohmer, H. (1978) Comparison of hearing threshold determined by auditory pathway electric responses and by behavioural responses. Audiology, Jul-Aug, 17(4):285-92.

Recanzone, G.H., Allard, T.T., Jenkins, W.M. and Merzenich, M.M. (1990) Receptive-field changes induced by peripheral nerve stimulation in SI of adult cats. Journal of Neurophysiology, May, 63(5):1213-25

Recanzone, GH; Schreiner, CE, and Merzenich, MM. (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. Journal of Neuroscience, Jan, 13(1):87-103.

Roberts, R.C. and Ribak, C.E. (1987) An electron microscopic study of GABAergic neurons and terminals in the central nucleus of the inferior colliculus of the rat. Journal of Neurocytology, Jun, 16(3): 333-45.

Robertson, D. and Irvine, D.R.F. (1989) Plasticity of frequency organization in auditory cortex of guinea pigs with partial unilateral deafness. J. Comp. Neurol. 282: 549-556.

Rubens, R.J. and Rapin, I. (1980) Plasticity of the developing auditory system. Ann. Otol. Rhinol. Laryngol. 89, 303-3111.

Salvi, R.J., Hamernik, R.P., and Henderson, D. (1978) Discharge patterns in the cochlear nucleus of the chinchilla following noise induced asymptotic threshold shift. Experimental Brain Research, 32(3):301-20

Salvi, R.J., Saunders, S.S., Gratton, M.A., Arehole, S., and Powers, N. (1990) Enhanced evoked response amplitudes in the inferior colliculus of the chinchilla following acoustic trauma. Hearing Research, 50(1-2):245-57.

Schindler, R.A; Kessler, D.K., and Barker, M. (1995) Clarion patient performance: an update on the clinical trials. Annals of Otology, Rhinology, and Laryngology. Supplement, Sep, 166:269-72.

Schwaber, M.K., Garraghty, P.E., and Kaas, J.H. (1993) Neuroplasticity of the adult primate auditory cortex following cochlear hearing loss. American Journal of Otology, May, 14(3):252-8.

Semple, M.N. and Aitkin, L.M. (1979) Representation of sound frequency and laterality by units in central nucleus of cat inferior colliculus. Journal of Neurophysiology, Nov, 42(6):1626-39.

Shannon, RV. (1992) Temporal modulation transfer functions in patients with cochlear implants. Journal of the Acoustical Society of America, Apr, 91(4 Pt 1):2156-64.

Shepherd, R.K., Matsushima, J., Martin, R.L., and Clark, G.M. (1994) Cochlear pathology following chronic electrical stimulation of the auditory nerve: II. Deafened kittens. Hearing Research, Dec, 81(1-2):150-66.

Shirane, M. and Harrison, R.V. (1991) The effects of long and short term profound deafness on the responses of inferior colliculus to electrical stimulation of the cochlea. Acta Oto-Laryngologica. Supplement, 489:32-40.

Sillman, J.S., LaRouere, M.J., Masta, R.I., Miller, J.M., and Nuttall, A.L. Electrically stimulated increases in cochlear blood flow: I. Frequency and intensity effects. Otolaryngology and Head and Neck Surgery, 1989 Apr, 100(4):308-16.

Snyder, R.L., Rebscher, S.J., Cao, K.L., Leake, P.A. and Kelly, K. (1990) Chronic intracochlear electrical stimulation in the neonatally deafened cat. I: Expansion of central representation. Hearing Research, Dec, 50(1-2):7-33.

Snyder, R.L., Rebscher, S.J., Leake, P.A., Kelly, K., and Cao, K. (1991) Chronic intracochlear electrical stimulation in the neonatally deafened cat. II. Temporal properties of neurons in the inferior colliculus. Hearing Research, Nov, 56(1-2):246-64.

Snyder, R; Leake, P; Rebscher, S; Beitel, R. (1995) Temporal resolution of neurons in cat inferior colliculus to intracochlear electrical stimulation: effects of neonatal deafening and chronic stimulation. Journal of Neurophysiology, Feb, 73(2):449-67.

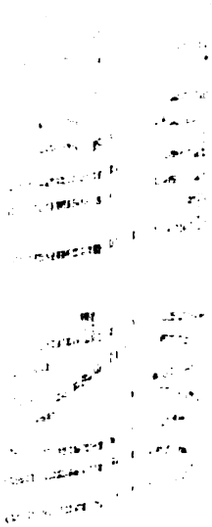
Snyder, R.L., Leake, PA., and Hradek, G.T. (1997) Quantitative analysis of spiral ganglion projections to the cat cochlear nucleus. Journal of Comparative Neurology, Mar 3, 379(1):133-49.

Snyder, R.L. and Sinex, D. (1998) Tonotopic reorganization of cat primary auditory cortex (AI) after acute lesions of restricted sectors of the spiral ganglion. 28th annual meeting, Society for Neuroscience, Los, Angeles California November 7-12.

Spoendlin, H. (1975) Retrograde degeneration of the cochlear nerve. Acta Otolaryngology. 79, 266-275.

Staller, S., Beiter, A., and Brimacombe, J. (1991) Children and multichannel cochlear implants. In H. Cooper (ed.), Cochlear Implants: A practical guide. San Diego, CA: Singular Publishing Group.

Staller, S.J., Dowell, R.C., Beiter, A.L. and Brimacombe, J.A. (1991) Perceptual abilities of children with the Nucleus 22-channel cochlear implant. Ear and Hearing, 1991 Aug, 12(4 Suppl):34S-47S.



Stryker, M.P. (1982) Role of visual afferent activity in the development of ocular dominance columns. Neurosciences Research Program Bulletin, Apr, 20(4):540-9.

Stryker, M.P. and Strickland, S.L. (1984) Physiological segregation of ocular dominance columns depends on the pattern of afferent electrical activity. Investi. Ophthalmol. Visual Sci. (Suppl.) 25:278.

Suga, F. and Hattler, K.W. (1970) Physiological and histopathological correlates of hereditary deafness in animals. Laryngoscope, Jan, 80(1): 81-104.

Szczepaniak, W.S. and Moller, A.R. Evidence of neuronal plasticity within the inferior colliculus after noise exposure: a study of evoked potentials in the rat. Electroencephalography and Clinical Neurophysiology 100: 158-164. 1996.

Trune, D.R. (1983) Influence of neonatal cochlear removal on the development of mouse cochlear nucleus. III. Its efferent projections to inferior colliculus. Brain Research, Jul, 285(1):1-12.

Volchan, E. and Gilbert, C.D. (1995) Interocular transfer of receptive field expansion in cat visual cortex. Vision Research, Jan, 35(1):1-6.

Vollmer, M., Snyder, R.L., Leake, P.L. and Rebscher, S.J. (1997) Factors influencing temporal and spatial representations of electrical signals in the inferior colliculus of deafened cats. (Abstr.) 20th Midwinter Meeting Assoc. for Research in Otolaryng.

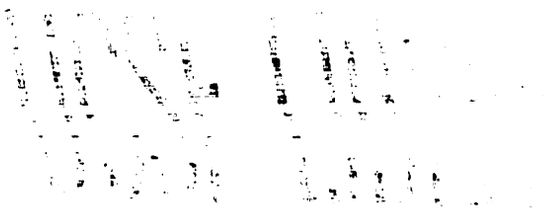
Vollmer, M., Snyder, R.L., Moore, C.M., Rebscher, S.J., and Leake, P.A. (1998) Processing of intracochlear electrical signals in the inferior colliculus (IC) of long term deafened cats (Abstr.) Assoc. Research Otolaryngol. 21:70.

Vollmer, M. (In Press) J. of Neurophysiology.

Vureck, L.S., White, M., Fong, M., and Walsh, S.M. (1981) Optoisolated stimulators used for electrically evoked BSER. Ann. Otol. Rhinol. Laryngol. 90: Suppl. 82:21-24.

Wall, J.T. and Kaas, J.H. (1986) Long-term cortical consequences of reinnervation errors after nerve regeneration in monkeys. Brain Research; 372: 400-404.

Wall, J.T., Huerta, M.F., and Kaas, J.H. (1992) Changes in the cortical map of the hand following postnatal median nerve injury in monkeys: modification of somatotopic aggregates. Journal of Neuroscience, Sep, 12(9):3445-55.



Wang, J., Salvi, RJ., and Powers, N. (1996) Plasticity of response properties of inferior colliculus neurons following acute cochlear damage. Journal of Neurophysiology, Jan, 75(1):171-83.

Webster, D.B. and Webster, M. (1977) Neonatal sound deprivation affects brain stem auditory nuclei. Arch. Otolaryngology. 103, 392-396.

Webster, W.R., Serviere, J., Batini, C., and Plant, S. (1978) Autoradiographic demonstration with 2[^{y}C] Deoxyglucose of requency selectivity in the auditory system of cats under conditions of functional activity. Neuroscience Letters 10: 43-48.

Weibel, E.R. (1979) Stereological Methods: Vol. 1, Practical Methods for Biologic Morphometry, Academic Press, New York.

Weinberger, N.M. (1997) Learning-induced receptive field plasticity in the primary auditory cortex. Seminars in Neuroscience 9, 59-67.

Willott, J.F. and Lu, S.M. (1982) Noise-induced hearing loss can alter neural coding and increase excitability in the central nervous system. Science, Jun 18, 216(4552):1331-4.

Willott, J.F. (1986) Effects of aging, hearing loss, and anatomical location on thresholds of inferior colliculus neurons in C57BL/6 and CBA mice. Journal of Neurophysiology, Aug, 56(2):391-408.

Willott, J.F., Parham, K., and Hunter K.P. (1991) Comparison of the auditory sensitivity of neurons in the cochlear nucleus and inferior colliculus of young and aging C57BL/6J and CBA/J mice. Hearing Research 53:78-94.

Willott, J.F., Aitkin, L.M., and McFadden, S.M. (1993) Plasticity of auditory cortex associated with sensorineural hearing loss in adult C57BL/6J mice. J. Comp Neurology 329:402-411.

Woolley, C.S., Gould, E., Frankfurt, M., and McEwen, B.S. (1990) Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. Journal of Neuroscience, Dec, 10(12):4035-9.

Xu, S.A., Shepherd, R.K., Chen, Y., and Clark, G.M. (1993) Profound hearing loss in the cat following the single co-administration of kanamycin and ethacrynic acid. Hearing Research, Nov, 70(2):205-15.

Xu, J. and Wall, J.T. (1997) Rapid changes in brainstem maps of adult primates after peripheral injury. Brain Research, Nov 7, 774(1-2):211-5.

Yang, L., Pollak, G.D., and Resler, C. (1992) GABAergic circuits sharpen tuning curves and modify response properties in the mustache bat inferior colliculus. Journal of Neurophysiology, Nov, 68(5):1760-74.

Zheng, J.L., Stewart, R.R., and Gao, W.Q. (1995) Neurotrophin-4/5, brain-derived neurotrophic factor, and neurotrophin-3 promote survival of cultured vestibular ganglion neurons and protect them against neurotoxicity of ototoxins. Journal of Neurobiology, Nov, 28(3):330-40.