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STUDIES ON AUDITORY AND VESTIBULAR END ORGANS AND BRAIN STEM NUCLEI

Harlow W. Ades, Principal Investigator

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STUDIES ON AUDITORY AND VESTIBULAR END ORGANS AND BRAIN STEM NUCLEI

Harlow W. Ades, Principal Investigator

This research program has been supported by the National Aeronautics & Space Administration for a period covering over nine years since the Principal Investigator came to the University of Illinois from the U. S. Naval School of Aviation Medicine (now the U. S. Naval Aerospace Medical Institute), Pensacola, Florida. The research has encompassed a broad spectrum of effort in the field of noise exposure and its effects. It has also contributed much to the understanding of the vestibular system as it affected the performance of the astronauts in the space program. The research has been a pioneering effort in the development of electron microscopy (both transmission and later scanning electron microscopy), and has shown otherwise unknown structures and formations in the anatomy of the inner ear and vestibular systems.

The NASA Technical Officers appointed as monitors of this grant over the years were as follows:

1965 - 1969: Walton L. Jones, NASA HQ

1970 - 1971: Randall Chambers and Phil Edge - Langley

1971 - 1973: David Winter, Ames Research Center

1973 - 1974: William Mehler, Ames Research Center

The Principal Investigator has served on numerous panels and committees for NASA and for many years was a member of the NASA Research Advisory Committee on Advanced Research and Technology (OART). In this regard he was appointed a member of the Italian National Research Council in 1968 and served until 1971. The Council met in Milano, Italy, and reviewed research being done by Dr. Torquato Gualtierotti at the Space Biology Laboratory in that city. Professor Ades spent three years as a member of the University of Illinois' Task Force on Noise in conjunction with the Illinois Pollution Control Board. As such he was instrumental in achieving the passing of the first Illinois statutes on the regulation of stationary noise. He is presently a member of

the Executive Council of National Academy of Sciences' Committee on Hearing and Bioacoustics (CHABA) after having served many years on subcommittees to CHABA on noise problems. Research results have been disseminated by the participation of the Principal Investigator and other members of the laboratory at many scientific meetings where papers have been presented (refs. 2, 5, 6, 9, 13, 16, 18, 27), including participation in each of the NASA Symposia (refs. 3, 4, 10, 15) which were held.

There have been joint efforts in this project involving laboratories that have mutual interests in the research, and in furthering the knowledge in the field. Some of them include The University of Gothenburg and the University of Uppsala in Sweden where Professor Dr. Hans Engström has participated extensively in the electron microscopy aspect of the program. Numerous joint authorships of publications in the forms of Books edited (refs. 19,25), book written (ref. 1), Chapters (refs. 18, 20, 21, 23, 24), and articles (refs. 7, 14, 17) have added to the literature in this field. Dr. W. D. Neff at Indiana University has had a joint project with Illinois in this same regard (refs. 16, 24, 28); Loyola University of Chicago with Dr. Terry Dolan (refs. 16, 28); Emory University, Yerkes Primate Center, with Dr. Geoffrey Bourne; and Naval Aviation Medical Institute with Dr. Ashton Graybiel.

As a result of these joint efforts, the Bioacoustics Research Laboratory, University of Illinois, has been host to quite a number of investigators who have spent anywhere from a few weeks to a year and a half in residence here, developing techniques and taking advantage of the particularly unique advantages available. Some of these included: Goran Bredberg, M. D. (University of Gothenburg and University of Uppsala) who spent one and a half years here (refs. 2, 5, 13, 14, 16, 17, 20, 22, 28); Dr. Henrik H. Lindeman (University of Gothenburg and University of Uppsala) who also spent one and a half years here (refs. 3, 14, 15, 17, 22); Ms. Berit Engström, (University of Uppsala) for one year and several shorter periods (ref. 25). One of our Illinois graduate students, Jerome Sugar spent three months studying in Dr. Engström's laboratory

in Uppsala. A direct result of his association with the two laboratories was his contribution of an article, "Stria Vascularis," which appeared in Inner Ear Studies (Ades and Engström, editors). Dr. Sugar was in his last year at Medical School at the time and is presently doing his residency with Dr. Harold Schuknecht at Massachusetts General Hospital in Boston. Another one of our graduate students, Charles W. Stockwell (refs. 10, 11, 12), completed his Ph.D. with Professor Ades as his advisor, was accepted in the U. S. Army and received orders to Naval Aerospace Medical Institute, Pensacola, where he worked for three years with a team there on noise effects and vestibular effects. He is now on the faculty at The Ohio State University in Columbus.

The research under this grant has furthered the knowledge and added expertise to the field of inner ear morphology, and the effects of noise stimulation on the organ of Corti; by its contribution to the training of graduate students who have gone into the field as a direct result of their courses and participation in the research programs offered by the Bioacoustics Research Laboratory. The Principal Investigator has appointments in three different departments at the University of Illinois (Electrical Engineering, Physiology & Biophysics, and Psychology),and through such affiliation, draws the special types of students who can make a definite contribution through their doctoral programs and research. He has been thesis advisor (and served on doctoral committees) to students from other departments such as Speech and Hearing, Biochemistry, and the School of Basic Medical Sciences. This means that each student has spent a period of concentrated study - anywhere from two to four years - on his graduate courses and the research that ultimately results in the material for the doctoral thesis and other publications (refs. 8, 11, 12, 14, 15, 19, 26, 27). During this time, as each student has completed the prescribed curriculum and written a report of the work, acknowledgment has been made to NASA for the support received through the grant to Professor Ades (NASA NGL 14 005 074).

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Manuscript copies of these two articles are appended.

SUMMARY

As stated briefly in the opening paragraph of this report, the support of this research project by NASA has enabled the Principal Investigator and his collaborators to add much to the field of knowledge of the organ of Corti and of the vestibular epithelia. It is stated much more to the point in the Introduction of the article authored by G. Bredberg, H. W. Ades, and H. Engström, "Scanning Electron Microscopy of the Normal and Pathologically Altered Organ of Corti," which appears in INNER EAR STUDIES. The following somewhat paraphrases that portion.

The authors have published a series of articles over a period of several years dealing with the morphology of the inner ear of animals and man. Many of those have been devoted to a systematic mapping of the sensory cells and nerve elements of normal and pathologically altered cochlear and vestibular epithelia. They were predicated initially on the idea of portraying properly the cell destruction caused by noise and ototoxic antibiotics. It was considered further that they would gain by the application of electron microscopic techniques. Transmission electron microscopy, while yielding illuminating insights on certain qualitative factors, did not give a quantitative estimate of the sensory cell population in normal or damaged cochleas, as it was known it would not. This left a need for a method of quantitating cells throughout the sensory organ, which in turn led to the development of the surface specimen method by which each sensory cell of the organ of Corti can be examined in situ and the entire organ mapped accordingly by light/phase contrast microscopy.

During the further development of the surface specimen method and its application to problems of noise exposure, the technique of scanning electron microscopy became available to us, and has proven to enjoy certain advantages over both light/phase contrast microscopy and transmission electron microscopy, though it does not supplant either. What it does in part is to bridge the wide gap between light microscopy and transmission electron microscopy. It is an additional adjunct to the study of the inner ear, and only by the judicious combinations of methods and their convergence on the same problems can progress be firmly made. Thus,

conventional transmission electron microscopy has been used widely and has contributed greatly to the knowledge of inner ear morphology. Likewise, light/phase contrast microscopy has clarified, both before and since, many interesting aspects of the morphology of bot cochlear and vestibular portions of the inner ear. Scanning electron microscopy has already had a considerable impact on this research, illuminating structures which were previously seen dimly or not at all. It does so by showing true three-dimensional pictures with a great depth of field and good resolution. It has added a new dimension to cochlear and vestibular morphology, which, while mainly in the realm of surface features, is applicable to a more limited degree to subsurface structures as well.

Most of the pictures presented in this paper represent some aspect of the normal morphology of the organ of Corti. They illustrate the three-dimensional structure in a new way that was made possible by the scanning electron microscope. There are a few figures, mainly transmission electron micrographs, which show the interrelationship and interdependence of transmission electron microscopy and scanning electron microscopy, or illustrate in a different way what is seen in a scanning picture. There are some few figures which are taken from pathological cochleas, or fetal cochleas, and are included to provide illustrations of a few things that can be done with scanning electron microscopy, and to indicate a few obvious areas of future research. This presentation in no way claims to give a complete picture of the morphology of the organ of Corti.

On this note, it should be pointed out that this laboratory is now operating on a very limited budget, and although there are several publications, either in press or in manuscript form ready to be submitted to journals, these will be acknowledged under the present grant from NASA, NGR 14 005 221 which covers the period of one year from January 1975 in the amount of $34,965. We are hoping to have continued support from NASA to complete results on data that have accumulated through the past several years of research.

Richard R. ALMON, Ph.D. 1971: "The Effect of Nerve Growth Factor upon Axoplasmic Transport in Sympathetic Neurons of the Mouse."

Roger W. WEST, Ph.D. 1970: "Electron Microscopy of Normal and Degenerated Ground Squirrel Retina." (See refs. 14 and 15) Thesis summary:

The synaptic organization of the inner plexiform layer of ground squirrel retina was studied using electron microscopy and related to its ganglion cell receptive fields. Other studies have shown that species with a majority of ganglion cells that respond optimally only to complex stimuli have more amacrine-amacrine and serial amacrine synapses and fewer bipolar-ganglion synapses than species with a majority of ganglion cells that respond well to simple stimuli. The present study found that ground squirrel retina, which has about equal numbers of both types of ganglion cells, also has numbers of synapses per unit area between those found in species with a majority of one or the other type of ganglion cell.

It has been reported that in ground squirrel all ganglion cells that respond optimally to complex stimuli project their fibers to the superior colliculus and all ganglion cells that respond well to simple stimuli project their fibers to the lateral geniculate. This made it possible to compare the synaptic inputs of ganglion cells isolated with respect to receptive field type. One group of ground squirrels was lesioned in the lateral geniculate and another group in the superior colliculus. Then, after allowing time for ganglion cell degeneration, the synaptic input of isolated populations of ganglion cells which responded best to complex or simple stimuli was studied.

The results indicated that both types of ganglion cells received nearly equal proportions of bipolar synapses but that ganglion cells which responded best to complex stimuli received at least twice as many amacrine synapses as did ganglion cells that responded well to simple stimuli.

1928

Inner Ear Damage and Hearing Loss after Exposure to Tones of High Intensity[1]

by

T. R. Dolan[2], H. W. Ades, G. Bredberg[3], and W. D. Neff

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[1] The research reported in this paper was supported by grants from the National Aeronautics and Space Administration, NGL 14-005-074 (Ades) and NGL 15-003-077 (Neff).

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Abstract

Experimental animals (cats) were exposed to tones of 125, 1000, 2000, and 4000 Hz at sound pressure levels in the range 120 to 157.5 dB, and for durations of one hour (1000, 2000, 4000 Hz) or four hours (125 Hz). Pure tone audiograms were obtained for each animal before and after exposure. Post-exposure tests were continued until complete recovery of hearing had occurred or until a stable permanent threshold shift had been measured. Cochleas of animals were examined by phase-contrast microscopy; condition of all hair cells was recorded. Extent of inner-ear damage and range of frequencies for which hearing loss occurred increased as exposure tone was decreased in frequency. For example, exposure to 4000 Hz produced damage in a restricted region of the cochlea and hearing loss for a relatively narrow range of frequencies; exposure to 125 Hz produced wide-spread inner ear damage and hearing loss throughout the frequency range 125 to 6000 Hz.

INTRODUCTION

Since the early days of experimental studies of hearing, investigators have used tones or noise of high intensity to stimulate the ear of an experimental animal and, thus, produce damage to structures and change in function of the inner ear. In a review of the literature " on the problem of stimulation deafness", Kemp in 1935 (—) cited 44 published reports of clinical and experimental investigations. The earliest clinical study mentioned that went beyond casual observations was published in 1890 (Habermann); the earliest experimental study, in 1907 (Wittmaack).

With the development of new techniques of examining inner ear pathology and of measuring inner ear function, important new information has come repeatedly through the use of this old procedure which allows physical characteristics of the exposure stimulus to be correlated with inner ear pathology; stimulus characteristics, with changes in inner ear function; and inner ear pathology, with changes in inner ear function.

Major advances in methods of detecting and mapping inner ear damage have included (in more or less chronological order):

1) improved techniques of sectioning and staining of mammalian cochleas; 2) graphic reconstruction of cochleas from serial sections; 3) surface preparation of organ of Corti and use of phase-contrast microscopy to examine and map all hair cells; 4) examination of selected regions of organ of Corti by transmission electron microscopy; 5) examination of cochlear structures by scanning electron microscopy.

The important methodological advances in studying inner ear function have been: 1) recording electrical responses produced by inner ear elements; 2) use of behavioral methods to obtain accurate measures of auditory discrimination (absolute and differential thresholds).

In the majority of published reports, inner ear damage has been assessed and related to frequency, intensity, duration and complexity of the sounds used to produce damage; or change in inner ear function as measured by electrophysiological or behavioral techniques has been related to physical aspects of the exposure stimulus. There have been a relatively small number of studies in which inner ear damage and inner ear function have been examined in the same animal after controlled exposure to sound stimuli. In the earliest of these experiments, inner ear pathology was assessed by examination of selected serial sections through the cochlea; graphic reconstructions were made according to the method of Guild.

Bredberg and Hunter-Duvar (in press) have recently published a summary of the results of animal experiments and human studies in which changes in hearing have been related to inner ear damage that was produced by exposure to high intensity sounds of by other means, such as surgery or administration of ototoxic drugs.

Through the use of the surface preparation of the cochlea and examination by phase-contrast microscopy, a more precise and complete picture of damage to the organ of Corti can be obtained. In a preliminary report, Dolan, Bredberg, Ades, and Neff (1970) described the results of an experiment in which the hearing of cats was tested before and after exposure to pure tones at high sound pressure levels. After sacrifice, surface preparations of the cochleas were made and all hair cells and adjacent structures were examined by phase-contrast microscopy. Details of this study, including results not available at the time of the preliminary report are given below.

METHODS AND PROCEDURE

The general scheme of the investigation included pre-exposure audiometric testing, controlled exposure to a potentially destructive stimulus, post-exposure audiometric testing, and postmortem examination of the inner ear.

Prior to initial training of the cats, one cochlea of each animal was destroyed by surgery. Additionally, the pinna of the experimental ear was removed and the surrounding area was reconstructed. Removal of the pinna was advantageous in that

it decreased the variance in intensity of acoustic stimuli at the ear drum due to positional changes of the pinna during behavioral testing. The absence of a pinna also allowed close examination of the ear drum prior to and following exposure, accurate monitoring of acoustic waveforms during exposure, and easy accessibility for cleaning of the external canal.

Exposure

For exposure to a high-intensity sound (tone), each experimental animal was anesthetized with diabutal and positioned in a stereotaxic instrument. Its head was held only by the bite bar. The appropriate stimulus frequency was generated by a low distortion General Radio oscillator. The output of the oscillator was fed through an Altec 1569A amplifier and into an Altec 802 D speaker. The output of the speaker passed through an adapter, consisting of telescoping brass tubes (this allowed for tuning by varying the length of the tube), and finally into a speculum that was inserted into the pinnectomized ear. The sound intensity was monitored through a 1 mm probe tube placed just beyond the orifice of the speculum; the probe was connected to a 1/2 inch Bruel and Kjaer microphone (Model 4134); the output of the microphone was amplified by a cathode follower amplifier (B&K 2615, 2801 power supply) and fed into a General Radio sound vibration analyzer. Sound and distortion data were calibrated with a B&K 4220 pistonphone and probe calibration coupler.

For the data presented in the Results section, frequencies of 125 Hz, 1000 Hz, 2000 Hz, and 4000 Hz were examined. At each frequency, the effects of sound pressure levels were also investigated.

Behavioral testing

Audiograms were obtained by an avoidance conditioning procedure. In order to avoid shock, the experimental animal was trained to cross from one side to the other of a double-grill cage when a series of tone pulses was presented. Each tone pulse had a duration of one second and rise and fall times of 300 msec each. The interval between pulses was one-half second. On any given trial, the tone pulses were presented for a period of ten seconds followed by shock given through the bars that formed the floor, sides, and top of the double grill cage.

Tone pulses were generated by a General Radio 1310-A oscillator, amplified by a McIntosh MC-250 amplifier, gated without respect to phase by an electronic switch (Olson and Ludwig, 1965), and presented via an Altec-Lansing 802 D speaker. The loud-speaker and double grill cage were located in a sound-deadened, double-walled room. Sound generating and control instruments were outside of the room. The experimenter sat at a control panel and could observe the experimental animal through a one-way window.

After an animal had learned to make avoidance responses to tones well above threshold level, the minimum sound pressure level (SPL) to which the animal would respond was determined

at each of the following frequencies: 125, 250, 500, 1000, 2000, 4000, 8000, and 12000 Hz. The general procedure for determining a "threshold" for a given frequency involved the attenuation of the tone by 15 B after each correct response. When a level was reached at which no response was made, the tone was increased by 15 dB and then attenuated in 5dB steps until another failure occurred. This procedure was repeated until the animal reversed his behavior from "responding" to "not responding" in the same 5 dB step twice in succession. Following determination of a "threshold" in this manner, the tone was increased by 50 to 80 dB and the entire procedure repeated. Two "thresholds" were obtained at a single frequency during each test session. Pre-exposure testing was continued until stable audiograms had been obtained, that is, until the range of threshold values at each frequency had decreased to 10 dB or less for several successive tests.

A similar procedure was followed after exposure to a high intensity tone. Audiograms were measured until recovery to normal pre-exposure levels occurred or until the amount of measured threshold shift remained constant for a period of several weeks. The time from exposure until sacrifice varied from two to four months for the animals included in the present report.

Post-mortem anatomical methods

The preparation of the cochleas for post-mortem examination was essentially similar to that described in detail by Engstrom,

Ades, and Andersson (1966), except that the entire organ of Corti was displayed on slides as surface specimens. All hair cells were counted and the results recorded on punch cards for computer analysis. The resultant cochleograms give a picture of the remaining or intact hair cells.

RESULTS

The behavioral data showing amount of hearing loss (PTS, permanent threshold shift) and the anatomical data showing the percent of hair cells missing as a result of the exposure to a tone of high intensity are summarized below for 16 animals. The amount of hearing loss suffered by an animal at a particular frequency is the difference in the median value of the final six measures obtained prior to the exposure and the final six measures prior to post-mortem examination.

Exposure to 4000 Hz

The behavioral and anatomical results for three animals exposed to a 4000 Hz tone are shown in Figure 1. The top half of Figure 1 shows the amount of hearing loss (relative to pre-exposure audiogram) as a function of frequency. The boxes in the lower half of Figure 1 show the percentage of normal hair cells remaining after the exposure as a function of the total cumulative number of hair cells measured from the basal end of the cochlea. Region and extent of absent or damaged hair cells are indicated by blackened areas. All three animals received an exposure of one hour duration. Cat LC 100 was exposed to a 135 dB SPL tone;* cats LC 76 and LC 110, to a 140 dB SPL tone.

As indicated in Figure 1, cat LC 100 suffered no hearing loss as a result of the exposure. Cats LC 76 and LC 110 had hearing losses in the frequency range 500 to 12000 Hz, with maximum loss occurring at the exposure frequency. At 4000 Hz, there was a permanent hearing loss of approximately 60 dB.

Figure 1 also shows that cat LC 100 sustained essentially no hair cell destruction as a function of the exposure. Only slight damage is indicated along the outer (3rd) row of outer hair cells (OHCs). Cats LC 76 and LC 110 had severe but narrow lesions in all three rows of OHCs and in the single row of inner hair cells (IHCs); lesions occurred in the upper basal turn about midway along the length of the cochlea.

Exposure to 2000 Hz

The results of exposure to a 2000 Hz tone that was one hour in duration are shown for three animals in Figure 2.

Cat RC 101 was exposed to a 130 dB SPL tone; it sustained a hearing loss in the range 1000 to 12000 Hz, with a maximum loss of about 38 dB occurring at the frequency of the exposure tone. No hearing loss was found for either the very low or very high frequencies. Cat LC 73, exposed to a 135 dB SPL tone, had greater hearing loss than RC 101 for all frequencies from 500 Hz to 12000 Hz. Again, the maximum loss, about 58 dB, occurred at the frequency of the exposure tone. Cat LC 67 was exposed to a 140 dB SPL tone; it had complete loss of hearing for frequencies from 2000 to 12000 Hz, severe loss at 1000 Hz, and smaller losses at 500 and 250 Hz.

The anatomical results of the exposures to a 2 kHz tone are given in the lower half of Figure 2. Cat RC 101, which had a 38 dB hearing loss at 2000 Hz, had only minor hair cell damage. With the exception of a very narrow lesion in which approximately 40% of the OHCs in the first row were missing, cat RC 101 appeared to have a nearly normal cochlea. Cat LC 73, which had a 58 dB hearing loss at 2000 Hz, had severe destruction of both inner and outer hair cells; the greatest damage to the outer hair cells occurred in two regions toward the apical end of the cochlea. One region of severe damage was in the approximate location of maximal activity caused by a 2000 Hz tone; the second region was at the apical tip of the cochlea and is apparently not reflected in the audiometric data. It should be noted that the lesion that occurred more distant from the apical end is much like the lesions found in cats LC 76 and LC 110 as a result of exposures to a 4 kHz tone; the lesion is confined to a very limited region of the basilar membrane.

The anatomical results of cat LC 67, which suffered the greatest PTS as a result of the 2000 Hz exposure, are also shown in Figure 2. As expected from the audiometric data, cat LC 67 had complete destruction of IHCs and all three rows of OHCs in all but the apical tip of the cochlea.

Exposure to 1000 Hz

The audiometric results for six animals exposed to a 1 kHz tone for one hour are shown in Figure 3. Exposure levels were 120, 130, and 140 dB. Cat LC 115 was exposed to a 120 dB SPL tone and had no PTS. Cat RC 97, exposed to a 130 dB

SPL tone, had a moderate amount of PTS at all frequencies above 250 Hz with the maximum loss again occurring at 2000 Hz. Cats LC 91 and LC 89 were both exposed to a 140 dB SPL tone and, after exposure, failed to respond to any signals between 250 and 20000 Hz. No audiometric tests were made at 125 Hz.

The anatomical results for the four animals exposed to 1000 Hz are shown in Figure 3. Cat LC 115 had essentially no damage as a result of the exposure. Cat RC 97 had a double-peaked lesion of the outer hair cells and a single narrow region of damage to inner hair cells. Cats LC 91 and LC 89 both suffered total destruction of OHCs and IHCs throughout all but the apical end of the cochlea.

Exposure to 125 Hz

The audiometric results for six animals exposed at 125 Hz for four hours are shown in Figure 4. Cat LC 96 was exposed to a 150 dB SPL tone and suffered no PTS. Cats LC 71 and LC 77 were exposed to a 155 dB SPL tone and, like LC 96, had normal post-exposure audiograms. Cat LC 105, exposed to a 157.5 dB SPL tone, had severe PTS for frequencies from 125 to 16000 Hz, the amount of PTS being greater at the high frequencies. Cats LC 88 and LC 70, which were exposed to intensities of 152.5 dB SPL and 155 dB SPL, respectively, failed to respond, after exposure, to tones throughout the range 125 to 16000 Hz.

The anatomical results for the animals exposed to 125 Hz are also shown in Figure 4. Cats LC 77, LC 96, and LC 71 suffered little or no damage from the sound exposure. Cat LC 105 had

complete loss of hair cells in the basal half of the cochlea and partial loss of cells in the upper turns. Cat LC 88 had almost total loss of OHCs and extensive damage to IHCs. Cat LC 70 had a nearly normal cochlea; limited damage occurred to outer hair cells at the apical end of the cochlea and to a small percentage of inner hair cells near the apex. There was also loss of OHCs in row 3 near the round window.

DISCUSSION

Locus of damage--frequency of exposure-tone

The relationship between frequency of exposure and locus of inner ear damage is most evident in those animals in which the exposure caused only a narrow region of damage. As a result of exposure at 4 kHz, for example, animals LC 76 and LC 110 sustained narrow but severe lesions of both OHCs and IHCs in the upper basal turn of the cochlea. In cases in which the exposure frequency was lowered to either 2000 Hz (LC 73) or 1000 Hz (RC 97), however, the region of hair-cell loss occurred at locations further from the basal end of the cochlea (approximately in the middle turn). In animals that suffered severe hair-cell destruction after exposure to either 2000 Hz or 1000 Hz (LC 67, LC 89, LC 91), all hair cells were destroyed from the upper middle or lower apical turn to the apical end of the cochlea.

Exposure to 125 Hz, in all but one case, produced either no damage (LC 77, LC 96, LC 81) or widespread destruction of hair cells (LC 105, LC 88) throughout the cochlea, extending from the basal turn to the apical end.

Inner ear damage - exposure level

For frequencies of 4,000, 2,000, and 1,000 Hz, the relationship between the amount of inner ear damage and the sound pressure level of the exposure tone is clearly seen: the extent of damage was, without exception, greater in those animals that received the highest exposure levels.

There was greater variability of results for exposure to 125 Hz; nevertheless, the two animals with greatest inner-ear damage were exposed to sound pressure levels as high or higher than the exposure levels of animals with less inner ear damage.

More interesting, perhaps, was the small amount of change in the exposure-intensity required to alter the resultant cochlear damage from minimal to severe. At all exposure frequencies investigated, for example, a 10 dB increase in the intensity of the exposure meant the difference between moderate or non-existant and severe destruction in the cochlea. At 125 Hz, a change of less than 5 dB in the exposure intensity sometimes meant the difference between a normal and a destroyed cochlea.

Hearing loss - exposure level

For exposures to 1000, 2000, and 4000 Hz, hearing loss increased with increase in sound pressure level. Again, there was greater variability in results for animals exposed to 125 Hz.

Hearing loss - region and extent of inner-ear damage

For animals exposed at 1000, 2000, and 4000 Hz, the frequency at which maximal hearing loss occurred corresponded well with the locus of hair-cell damage. Often, however, the range of frequencies for which hearing loss occurred did not correspond well with the width of the cochlear lesion (particularly with narrow

cochlear lesions). Animals with maximal hearing loss at 4000 Hz (LC 76 and LC 110), for example, had damage to both IHCs and OHCs in the upper basal turn of the cochlea. Animals with maximal hearing loss at 2 kHz (RC 101 and LC 73) had hair cells destroyed in the middle turn of the cochlea (although LC 73 had a second lesion near the apex). The animal with maximal hearing loss at 1 kHz (RC 97) also had lesions in the middle and apical turns. In each of these cases, the range of frequencies at which hearing loss occurred was greater than would have been expected based on the width of the cochlear lesion or lesions. There are several possible explanations of this discrepancy. The most likely is that the histological data presented above are based primarily on a determina- of the apparent "presence" or "absence" of each hair cell using phase-contrast microscopy. No further attempt was made to ascertain the condition of a hair cell. More subtle alterations of a hair cell than its elimination or severe distortion would not be seen although other techniques such as electron microscopy might have revealed damage sufficient to render the cell non-functional. This explanation would also account for such discrepancies as represented by RC 101 which had considerable hearing loss but only minimal hair cell damage. The lesions sustained at the apical end of the cochleas of LC 73 and RC 97 may have been produced by interruption of the blood supply to the apical region, a disorder that might be unrelated to the sound exposure (see Bernstein and Schuknecht, 1967).

In all cases in which the exposure caused widespread destruction of OHCs and IHCs (LC 105, LC 88, LC 91, LC 89, LC 67),

there was a corresponding severe loss of hearing at all frequencies or complete loss at higher frequencies and severe loss extending into the lower frequency-range. In one case, LC 70, the post-exposure hearing loss cannot be explained in terms of inner-ear damage.

Hearing loss - destruction of IHCs or OHCs?

The data obtained in this experiment do not permit any definitive answer to the question: Does the audiogram reflect the condition of inner hair cells, outer hair cells, or both? Evidence from several animals are relevant to this question. Cat LC 88 had almost complete destruction of OHCs, but a large number of IHCs in the apical turn were still present; post-exposure testing indicated complete loss of hearing. LC 91 also had complete loss of hearing although some IHCs remained in the apical and basal turns. RC 101 had post-exposure hearing loss for frequencies from 2000 to 10,000 Hz although IHCs remained throughout the cochlea.

The above cases (LC 88, LC 91, and RC 101) might be taken as evidence that the audiogram does not necessarily reflect the condition of the IHCs.

In contrast to the above cases, LC 73 had normal hearing for low frequencies despite the nearly total destruction of OHCs in the apical turn; IHCs were intact in the apical turn and throughout the cochlea except for a narrow region in the middle turn. In this case, it might be argued that the audiogram does reflect the condition of the IHCs.

Finally, in considering the results of the present study and of many others that have related hearing loss to inner-ear damage,

the limitations of methods used in assessing inner-ear damage must be kept in mind. No single histological technique provides a complete assessment of intracochlear damage or change resulting from an exposure. It is necessary to accurately assess damage to hair cells, intracochlear changes such as mechanical destruction of nerve fibers, disruption of the synaptic region between sensory cell and nerve fiber, interruption of blood supply, and damage to supporting structures.

There are also limitations in the methods used to measure changes in hearing in experimental animals. Occasional anomolous results may be expected. In an animal with a severe hearing loss, it may be difficult to obtain threshold measurements. For a normal animal or one with moderate hearing loss, tests of absolute threshold may be started by presenting a tonal signal well above threshold and decreasing it until the animal no longer makes a behavioral response. For animals with severe hearing loss, it may not be possible to use a tonal stimulus that is much above threshold. Therefore, great care must be taken not to produce "neurotic" behavior by creating highly stressful conditions for an animal--particularly one that has been exposed to loud sound or has otherwise been treated so as to produce a severe hearing deficit. The presence of tinnitus may also disrupt the animals performance in response to weak tonal signals.

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SUMMARY

Experimental animals (cats) were exposed to tones of 125, 1000, 2000, and 4000 Hz at sound pressure levels in the range 120 to 157.5 dB, and for durations of one hour (1000, 2000, 4000 Hz) or four hours (125 Hz). Pure tone audiograms were obtained for each animal before and after exposure. Post-exposure tests were continued until complete recovery of hearing had occurred or until a stable permanent threshold shift had been measured. Cochleas of animals were examined by phase-constrast microscopy; condition of all hair cells was recorded. Extent of inner-ear damage and range of frequencies for which hearing loss occurred increased as exposure tone was decreased in frequency. For example, exposure to 4000 Hz produced damage in a restricted region of the cochlea and hearing loss for a relatively narrow range of frequencies; exposure to 125 Hz produced wide-spread inner ear damage and hearing loss throughout the frequency range 125 to 6000 Hz.

Figure 1. Pure tone thresholds (upper half of figure) and plots of inner ear damage (lower half of figure) for animals exposed to 4000 Hz tone. For each animal, the O-dB line represents its preoperative hearing level. Hearing loss or permanent threshold shift (PTS) is plotted as a deviation from the O-line.

In the plots of inner ear damage, the base line (labeled 0,4,8,12 etc) indicates the number of hair cells, as counted starting at the base of the cochlea. For example, 4 means that to that point, four hundred hair cells had been counted. The number of destroyed or damaged hair cells is shown by the black-shaded areas. For example, in the plot for Cat LC 110, all of the hair cells in outer row 3 were damaged in the region occupied by hair cells 1400 to about 1600 (upper basal turn). The damage was less in the other two rows of outer hair cells and in the single row of inner hair cells.

NR= No response to tone at maximum level available.

Figure 2. Pure-tone thresholds and inner ear damage of animals exposed to 2000 Hz (Figure 1 for explanation)

Figure 3. Pure-tone thresholds and inner ear damage for animals exposed to 1000 Hz (Figure 1 for explanation)

Figure 4. Pure-tone thresholds and inner ear damage for animals exposed to 125 Hz. (Figure 1 for explanation.)

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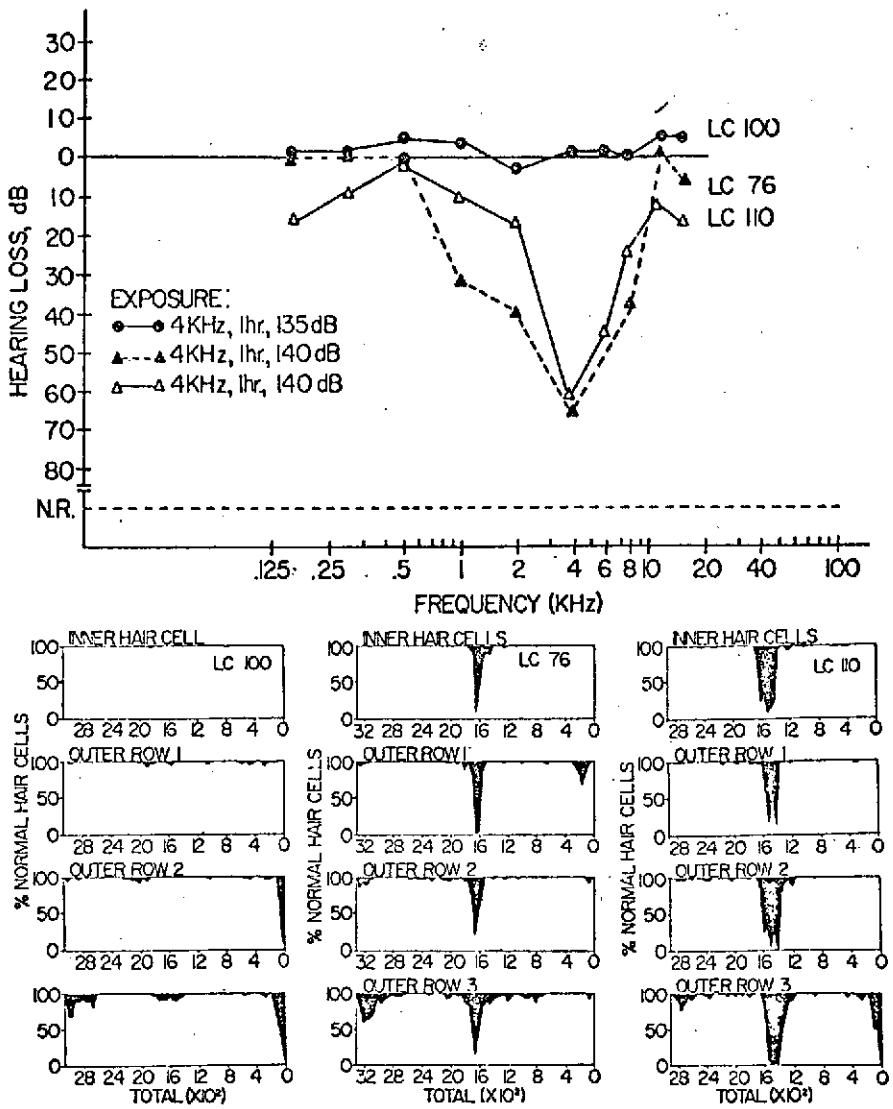

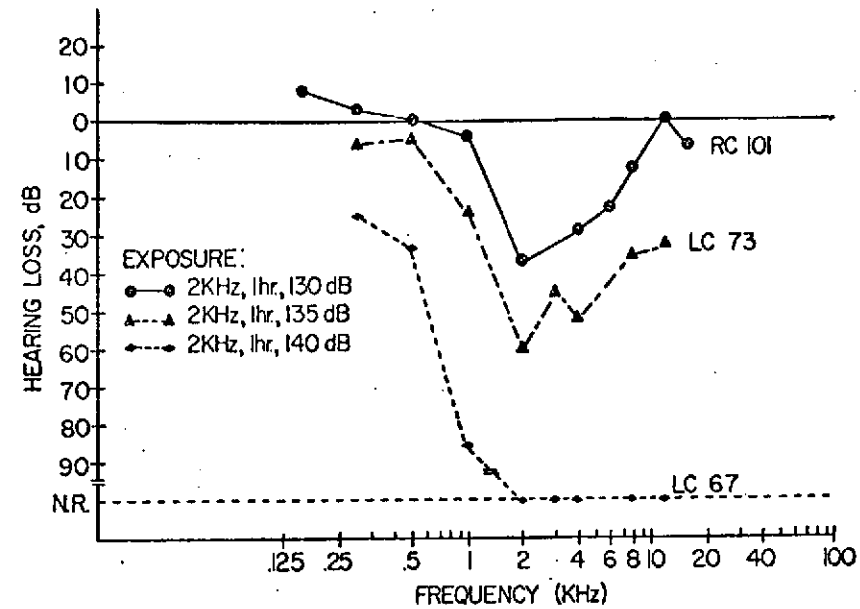

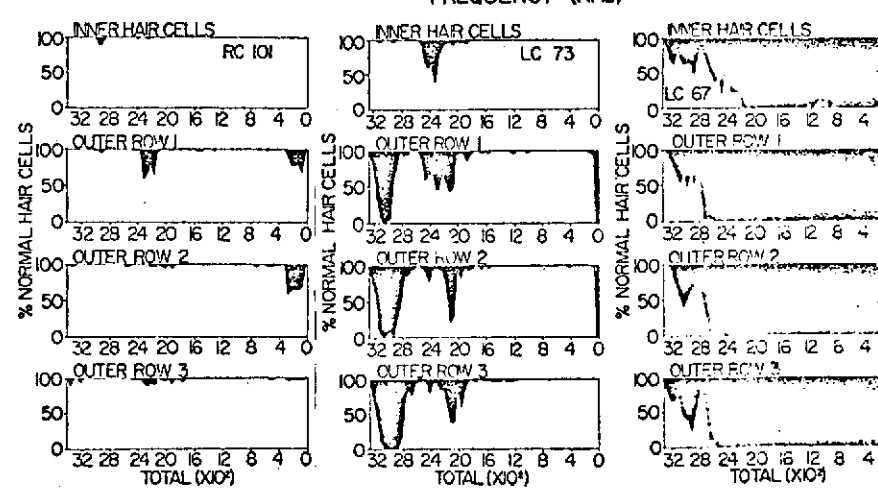

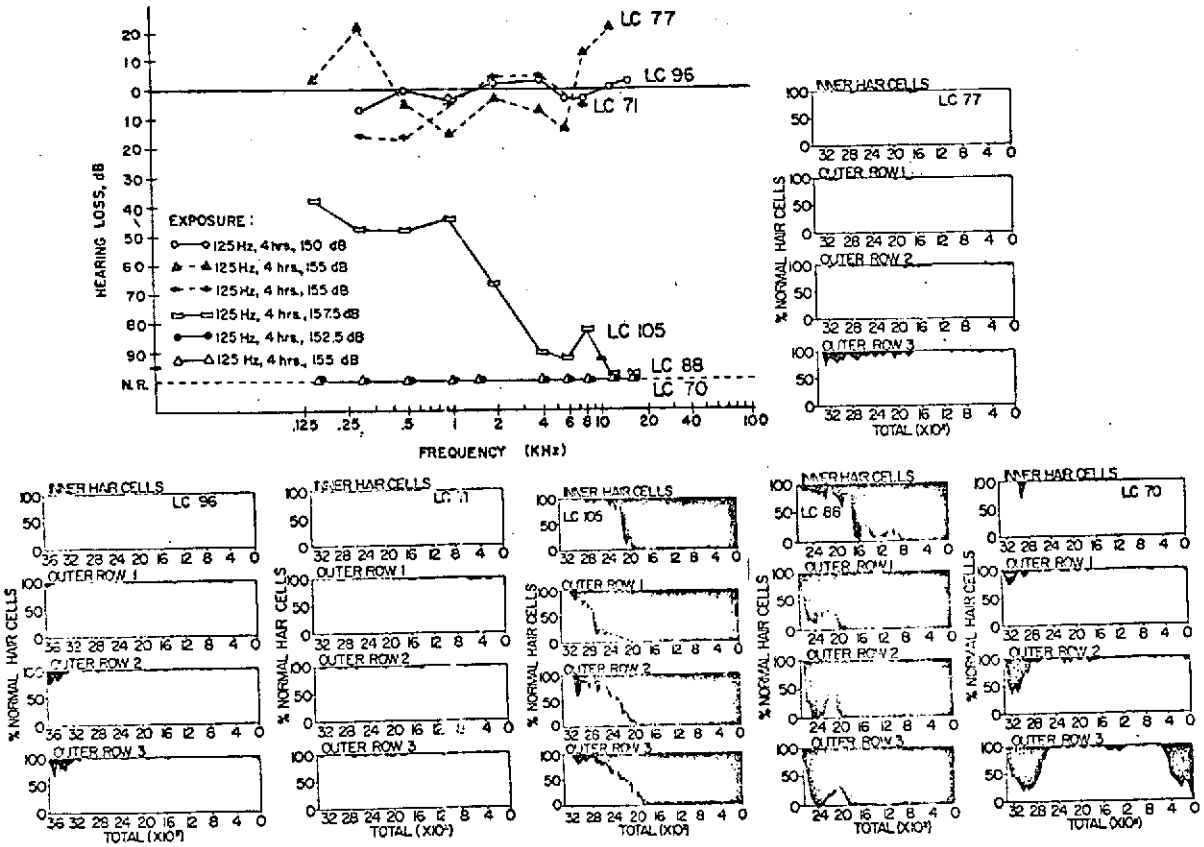

Charles W. STOCKWELL(10, 11, 12) Ph.D. 1968: "Patterns of Hair Cell Damage in the Guinea Pig after Intense Auditory Stimulation." (Department of Otolaryngology, Vestibular Physiology, The Ohio State University, Columbus)

PATTERNS OF HAIR CELL DAMAGE AFTER INTENSE AUDITORY STIMULATION

SUMMARY

Guinea pigs were exposed to pure tones of 125, 500, 1000, 2000 and 4000 Hz at intensities of 130 and 150 dB SPL for a period of one or four hours. Each cochlea was prepared histologically using the "surface specimen technique," and a cochleogram of the sensory cell population was constructed to show the pattern of hair cell loss.

The radial distribution of damage was related to exposure frequency. Lower frequencies produced proportionally greater damage in distal hair cell rows than did higher frequencies. Hair cell damage caused by exposures at 150 dB was severe over wide areas, extending from the supposed site of maximum stimulation primarily toward the base. Exposures to 130 dB caused damage which was more selective than has been reported previously. Lesions produced by a 4000 Hz tone appeared near the stimulation maximum for that frequency, but lesions caused by lower frequencies tended to appear progressively nearer the base with respect to stimulation maxima. The existence of multiple peaks of damage was a prominent feature.

Two mechanisms of damage appear necessary to interpret the damage patterns which were observed: 1) direct mechanical stress, which was most important at higher frequencies, and 2) gradually accumulating effects, which were most important at lower frequencies, and to which OHC were particularly susceptible. Particular attention was paid to the possible role of the ear's nonlinearity in accounting for discrepancies between hair cell damage patterns and patterns of normal hair cell stimulation.

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* Ref. 12

Cochlear Hair-Cell Damage in Guinea Pigs after Exposure to Impulse Noise

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Fourteen young guinea pigs were individually exposed to 500 rounds of paper "caps" fired in a toy gun at a distance of 30 cm. The gun was fired at intervals of 1–5 sec. After seven weeks survival, the cochleas were removed and each complete organ of Corti examined, using Engström's surface-preparation technique. Cochleograms (tabular forms showing position and condition of each cell) were prepared for each row of hair cells. Curves were plotted showing number of damaged cells as a function of distance from the base. In approximately 80% of the cases, total destruction of hair cells occurred in a narrow band midway along the organ of Corti. The severity and distribution of damage for the group are compared with similar data for another group, which was exposed to high-intensity pure tones.

The immediate implication of the data presented is fairly obvious. Repetitive impulse noise of the type and level indicated is sufficient to produce marked damage to cochlear hair cells in the guinea pig. It can be assumed that the human ear would be similarly affected, though to what degree is not predictable because of the presumptive difference in vulnerability between the two species. No estimate can be made, on the basis of these data, of the permanent hearing loss that would result from such exposure. The correlation between hair-cell damage and hearing impairment is largely unknown for either species.

It was found that the damage pattern due to cap-gun exposure was closely similar to that produced by a continuous exposure to a tone of 2000 Hz at 125–130 dB for 4 h, with respect to both degree of damage and site of damage along the organ of Corti. This is contrary to the widely held opinion that impulse noise produces hair-cell damage that is most severe at the base of the cochlea. Furthermore, the damage encountered in the present experiments did not exhibit the wide variability often reported. It was consistently focalized in the second turn of the cochlea. The region is the one that has been assigned traditionally to the reception of 2–4-KHz tones, suggesting a correlation with the 4-KHz notch in the human audiogram that is often seen in cases of presumptively noise-induced hearing loss; however, this interpretation must be drawn with caution. More extensive studies of hair-cell damage from pure-tone exposure make it apparent that the relation between exposure tones and locus of damage is not the simple one that is often tacitly assumed by derivation from place theory. A separate report of experiments relative to that question is in preparation and will be published soon.

It is clear that the guinea pig suffers extensive hair-cell loss from exposures of the type described. While there is probably some difference in damage threshold between guinea pig and man, it seems fair to assume that the ears of humans may be affected similarly, though possibly in different degree. Consequently, in any effort to account for the notch type of hearing loss that is often encountered, even in young people, it becomes necessary to consider noisy toys as a potential factor in addition to the many other sources of potentially damaging noise. It is not surprising to learn that, in his studies of histopathology in the human ear, Bredberg (1968) was compelled to derive his normative data from late fetal and neonatal ears. All of the postnatal ears, even those from children, showed some degree of hair-cell loss, and, by the latter part of the second decade of life, this had assumed substantial proportions. This is not to say that such findings are to be interpreted as due in any particular degree to early exposure to noisy toys, but they do call into question the norms to which human hearing thresholds are commonly referred.

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The following lists the names of some of the students; dates of obtaining the degrees; theses titles; and ultimate placement where applicable. Summaries are included as a sample of their work.

Mary Jayne CAPPS(8) Ph.D. 1967: "Auditory Frequency Discrimination after Transection of the Olivo-Cochlear Bundle in Squirrel Monkeys." (Present address: Department of Otolaryngology, University of Minnesota Medical School, Minneapolis, Minn.)

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Auditory Frequency Discrimination after Transection of the Olivocochlear Bundle in Squirrel Monkeys

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Received February 4, 1968

Difference limens for auditory frequency discrimination were determined before and after transection of the olivocochlear bundle in four squirrel monkeys (*Samiri sciureus*). A modified Wisconsin General Test Apparatus was used to determine the frequency difference limens at 1000 and 4000 cycle/sec. Following transection of the olivocochlear bundle, all of the animals showed marked deficits in frequency discrimination performance. The mean absolute difference limens (75% correct responses) were increased by 450 cycle/sec at 1000 cycle/sec and by 600 cycle/sec at 4000 cycle/sec. Performance on an auditory localization task was not affected by the lesions of the efferent tract. It is suggested that the efferent auditory system operates to sharpen the frequency resolving power of the ear.

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Scanning Electron Microscopy of the Organ of Corti

Abstract. *With the scanning electron microscope we have examined normal cochlear sensory epithelium of the guinea pig and cat and that damaged by noise. The studies demonstrate how the regular surface architecture of the organ of Corti is altered after exposure to noise. The changes include loss of sensory hairs, formation of giant hairs, and complete degeneration of circumscribed areas of the organ of Corti. Our method greatly reduces the artifacts.*

Earlier scanning electron microscopy of the inner ear has shown considerable artifacts due to shrinkage and compression. The use of the freeze-drying technique greatly reduces the artifacts so that a more normal shape of the structures is preserved. The scanning electron microscope will no doubt become a useful tool in the study of the organ of Corti, and be of special value for the understanding of spatial relations and the pattern of innervation inside the organ. The effects of noise and ototoxic drugs on the organ of Corti and other degenerative changes will provide additional areas of study.

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SUPPLEMENT 301

Inner Ear Studies

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CONTENTS

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Scanning Electron Microscopy of the Organ of Corti after Intense Auditory Stimulation: Effects on Stereocilia and Cuticular Surface of Hair Cells*

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Received March 18/June 20, 1972

Summary. The organ of Corti of cats, guinea pigs and chinchillas exposed to pure tones of high intensity were first mounted on a slide in glycerol and mapped with light/phase contrast microscopy. Subsequently the same specimens were rinsed in water, freeze-dried and prepared for scanning electron microscopy. The study shows that it is well possible to use the same preparation for light microscopic and scanning electron microscopic investigations. Scanning electron microscopy permits detailed studies of the surface topography of the organ of Corti and demonstrates structural changes not visible in light/phase contrast microscopy. The changes are found on both inner and outer hair cells and include disarrangement of hairs, fusion of stereocilia and the formation of giant hairs, which exceed the normal stereocilia in length and thickness. The giant hairs are seen most frequently in cats following low frequency exposure. Moreover, the cuticular plates of the sensory cells are often extensively deformed. It is doubtful if these cells, which may look normal as studied by light/phase contrast microscopy, are still functional. In areas showing epithelial damage an increase in the number and size of the microvilli of the Deiters cells is often found.

* This work was supported by the National Aeronautics and Space Administration, NASA Grant: NGL 14005074 while the authors were under appointment to the Bioacoustics Research Laboratory at the University of Illinois, Urbana, Illinois, U.S.A. The authors would also like to acknowledge the cooperation of Professor B. Vincent Hall, Head of the Center for Electron Microscopy at that institution.

*Ref. 27

Comparison of critical ratios and critical bands in the monaural chinchilla*

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(Received 12 June 1974; revised 4 September 1974)

The major purpose of this study was to compare measures of critical ratios and critical bands in monaural chinchillas. Critical ratios were obtained from two groups of chinchillas tested at 0.5, 1, 2, and 4 kHz in each of three levels of broad-band noise. Critical bands were obtained by assessing the values of masked threshold in various narrow bands of noise centered on each test frequency. The measures of critical ratios and critical bands were found not to be parallel as a function of frequency, as is the case for human subjects. The present data were compared to those reported for the monkey and were found to be in essential agreement.

V. SUMMARY

Experiments designed to obtain the relationship between the critical ratio and the critical bandwidth in the chinchilla constitute the major portion of this investigation. The assumption that the critical bandwidth is 2.5 times (4 dB) larger than the critical ratio was supported at frequencies of 0.5 and 1 kHz. However, the expected relation between the critical ratio and the critical bandwidth was not found at 2 and 4 kHz. At these test frequencies, the difference between the critical ratio and critical bandwidth were 0 dB at 2 kHz and 1 dB at 4 kHz. It was also shown that these results were very similar to those obtained in a study using monkeys rather than chinchillas. At this time, no explanation can be offered for the discrepancies found when comparing data obtained in human experiments to those data obtained in studies using animal subjects.

The major findings of this investigation are:

(1) Manipulation of false-alarm rate induced a 1 to 4-dB difference in critical-ratio size.

(2) The band-narrowing technique appears to be an adequate method for obtaining critical bands in a free field using monaural chinchillas as subjects.

(3) The function relating direct and indirect measures of critical bandwidth appears to depend upon unknown subject variables. Specifically, the increase in the critical ratio as a function of test frequency is greater for the chinchilla than it is for man.

ACKNOWLEDGMENTS

We are indebted to Dr. Gordon Z. Greenberg, Dr. John J. O'Neill, and Dr. Frederic L. Wightman for their comments and criticisms. Dr. Harlow W. Ades donated the animals for this investigation and the surgery was performed by Dr. Aulikki Kokko-Cunningham and Patricia McDowell. The computer analysis for part of this investigation was accomplished by Dr. Aaron Averbuch. This research was supported by a grant from the National Aeronautics and Space Administration, number NGL 14 005 074 (Dr. H. W. Ades, Principal Investigator).

*This research was reported at the 84th Meeting of the Acoustical Society of America, Miami Beach, Florida, 1972.

†This study was submitted by W. H. Seaton to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Arts. The assistance and encouragement received from Dr. Constantine Trahiotis, thesis advisor, and Dr. Gordon Z. Greenberg and Dr. John J. O'Neill, committee members, is deeply appreciated.

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Comparison of critical ratios and critical bands in the monaural chinchilla*

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Subject Classification: 65.58, 65.75; 80.50.

INTRODUCTION

In 1940, Fletcher proposed the critical-band concept to account for data obtained in certain masking experiments. The concept is now extremely important for an understanding of auditory frequency resolution. Hence, it is relevant to many aspects of hearing, including perception of pitch and loudness, frequency discrimination, musical consonance and dissonance, poststimulatory fatigue, and discrimination of harmonic content of complex signals.

Scharf's (1970) review of the critical-band literature emphasizes the constancy of various behavioral measures of frequency resolution. Two of the methods are of concern here. One—an indirect method—yields an *estimate* of the critical bandwidth and is called the critical ratio. To obtain the value of the critical ratio, one simply determines the threshold of detectability of sinusoidal signals presented in a background of broad-band Gaussian noise. Adopting Fletcher's assumption that the signal is detectable when the power in the signal is equal to the power in the critical band, one needs only to subtract the spectrum level of the masker from the level of the threshold signal. Data obtained in many laboratories suggest that the size of the critical ratio is directly proportional to signal frequency for tonal signals above approximately 800 Hz (Scharf, 1970).

The second method of primary concern for this investigation is considered a "direct" method and requires the subject to detect tonal signals in various bandwidths of Gaussian noise. Data from these experiments show that masked thresholds remain essentially constant in bands of noise that are of "critical" width or wider. However, masked thresholds decrease in bands of noise that are narrower than the critical width.

Possibly because of the ease with which the critical ratio can be measured, most investigators have utilized the indirect method to estimate critical bandwidth in animals. Gourevitch (1970) collated critical-ratio data from studies on porpoises (Johnson, 1968), rats (Gourevitch, 1965), chinchillas (Miller, 1964), cats (Watson, 1963), and man (Hawkins and Stevens, 1950), and showed these critical-ratio functions to be similar to one another. The animal critical ratios as a function of frequency are roughly parallel to man's, with the porpoise and man having the smallest critical ratio, followed by those measured in the cat, chinchilla, and the rat in order of increasing size. The data indicate that these animals need a larger signal-to-noise than man in order to detect the presence of a pure tone in a background of broad-band noise. One implication of these parallel functions is that the critical-band mechanism in animals is similar to that of man. A second is that, because these animals have larger critical ratios, they also have larger critical bands and, therefore, poorer frequency-resolving ability than man. Also, these data have been interpreted to mean that the various measures of frequency resolution are related in a manner that is consistent *across* species.

For humans, the indirect (critical ratio) and direct (critical band) measures are simply related: the critical band is about two-and-one-half times greater than the critical ratio over a frequency range of approximately five octaves (Zwicker *et al.*, 1957). Since the critical-ratio data are similar across species (the functions are roughly parallel), it is tempting to assume that the relationship between the critical ratio and the critical band is also similar across species, i.e., that in animals as well as in man the critical band is two-and-one-half times larger than the critical ratio. One portion of this study was directed at this issue. Critical bandwidths were measured in chinchillas by one of the direct methods—so that they could be compared with critical-ratio measures.

The psychophysical procedure (modified method of limits) used in obtaining most critical ratio data from animals, however, does not permit separation of behavioral criterion and auditory sensitivity (Miller *et al.*, 1963). Animals are typically trained in a manner that establishes a very conservative decision rule resulting in low occurrences of false-alarm responses (for example,

see Schusterman, 1974). The extent to which the obtained differences in estimates of the critical ratio across species may be accounted for by differences in criteria is unknown.[1]

The second portion of this investigation included varying a reinforcement parameter to determine the effect on the critical-ratio estimate.[2] It was expected that an increase in false-alarm rate would result in a "lower" threshold and smaller critical ratios. In addition, it seemed possible that an unbiased measure of sensitivity could lead to a "better" measure of critical ratio because it would be unaffected by fluctuations in response criterion.

The motivation for making these comparisons is quite simple and direct. Results of auditory experiments performed on animals are often used to make inferences about the human auditory system (Greenwood, 1961b). To the degree that the critical-band measures depart from what is known about human performance, one may have to qualify many generalizations, including the notion that a cochlea found in one animal is a scale version of a cochlea found in another (von Békésy, 1960, pp. 500–510). While this particular realm of audition is richly endowed with data that permit and, indeed, encourage extrapolation across species, we believed that more detailed information would enable one to do so on a firmer foundation.

I. GENERAL METHOD

A. Procedure

1. *Training*

Six chinchillas with left cochlea surgically destroyed served as subjects. The animals were trained in quiet using an avoidance technique similar to that employed by Miller (1970). Each animal was given a 2-sec, 1-kHz tone pulse at about 75 dB SPL every 2 min. One 2-sec tone pulse made up a trial, ten trials were given in a session, one session was given per day, and animals were trained seven days a week. If an animal did not respond by crossing the barrier in the cage by the time the tone pulse was terminated, a loud buzzing sound and brief pulses of shock were presented simultaneously until a response was made. This procedure continued until each animal avoided shock on at least 70% of the trials. Then the intertrial intervals were changed from 2 min to a random sequence with a mean of 19.7 sec[3], and the measurement of thresholds at 1 kHz was begun. After a few thresholds had been measured at 1 kHz, the stimulus presentation was changed from a 2-sec pulse to three 750-msec pulses with a 250-msec interpulse interval. Additional test frequencies were introduced until the four test frequencies used for the study—0.5, 1, 2, and 4 kHz—had been run several times for practice. Two groups of three animals each were trained in a manner expected to produce differential rates of false-alarm responses—a false-alarm response being defined as the animal jumping over the barrier during any intertrial interval. This was done in the following manner. When threshold measurements were begun, the high-false-alarm animals (group I) received shock whenever they did not respond to tones that were near their threshold, while the low-false-alarm animals (group II) received only a buzz when they did not respond to tones near their threshold. This procedure was followed throughout all of the threshold measurement sessions of the training period for all of the test frequencies. At this point, only gross estimates of an animal's threshold were used. These estimates were based upon threshold measures that had been obtained in previous experiments conducted in this laboratory (Ades *et al.*, 1974).

2. *Threshold measurements*

Thresholds were measured using a modified method of limits. A sequence of trials was used to obtain the threshold value. On the first trial of the sequence, three 750-msec tone pulses were presented at an intensity level of about 75 dB SPL. If the animal responded, the intensity was reduced by 20 dB for the next trial. This reduction of the stimulus intensity in 20-dB steps was continued until the animal failed to respond. Then the intensity was raised 10 dB. If the animal did not respond, the trials were terminated for that frequency. If the animal did respond when the intensity was raised 10 dB, the the intensity was lowered in 10-dB steps until the animal failed to respond. Then the trials were terminated for that frequency. The threshold was defined as the average of the lowest intensity at which the animal responded and the highest intensity at which the animal failed to respond.

II. MEASUREMENT OF CRITICAL RATIOS

A. Apparatus

A film reader with a pre-punched tape was used to control an R–C timing circuit with relays. This timing circuit controlled an electronic switch that produced a gated output with a 50-msec rise/decay time. A sinusoid was fed to the electronic switch from an oscillator whose out-out was monitored by a frequency counter. The output of the electronic switch was fed through an attenuator to a resistive mixer. The output of a thermal noise generator passed through a second attenuator before being led to the resistive mixer. Mixer outputs were amplified and sent to a loudspeaker through an impedance-matching transformer.

B. Sound-field measures

The sound field was measured at 24 positions within the experimental cage using a condenser microphone and wave analyzer. These measurements showed the sound field was relatively flat up to about 10 kHz and then dropped off rapidly. Three levels of broad-band were employed. In terms of the electrical signals applied to the loudspeaker, the spectrum levels were equivalent to 5, 15, and 25 dB SPL. Because of minor nonuniformities in the power spectrum measured in the sound field, each spectrum level used to calculate the critical ratio was determined in the test cage by monitoring the noise through octave-band filters centered at each of the test frequencies. These measures were felt to be accurate as they varied across frequency in the same manner as did the sound-pressure level of pure tones of constant-voltage input to the loudspeaker.

TABLE I. Mean critical ratios and corresponding standard deviations obtained in critical ratio experiment. The data are reported for the high-false-alarm group (I), low false-alarm group (II), and I and II combined.

	Frequency in kilohertz							
	0.5		1		2		4	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Group I ($N=3$)	16	1.7	18	3.6	24	4.2	29	4.2
Group II ($N=3$)	17	5.7	21	4.6	25	2.0	33	4.3
Group I and II ($N=6$)	16	4.1	19	4.3	25	3.3	31	4.7

C. Masked thresholds in broad-band noise

Both the test frequencies and the order in which the animals were tested were randomized. In each session the thresholds at each test frequency were measured in three levels of wide-band masking noise. Subjects were tested twice a day, seven days a week. Six threshold measures were taken at each of the test frequencies for each spectrum level. Then critical ratios were calculated for each of the three spectrum levels for each subject and then averaged across subjects separately for the high- and low-false-alarm rate groups.

D. Results

Table I shows the critical ratios obtained from the critical ratio experiment. The results show that the high-false-alarm group (group I) produced critical ratios that were *slightly* smaller than the critical ratios of the low-false-alarm group (group II).

E. Discussion

Earlier it was suggested that the critical ratios reported by Gourevitch (1970) may have been "inflated" because the animal subjects were trained to maintain a low-false-alarm rate. We expected that if animals were trained to have a high-false-alarm rate, a smaller critical ratio could be obtained than if they had been trained to maintain a low-false-alarm rate. The data in Table I suggest that the differences in training may have produced a difference in critical-ratio measures. But, again, the differences are very slight, averaging about 2 dB. Reed and Bilger (1973) report differences of the same magnitude in critical-ratio measures obtained from humans tested in criterion-free and criterion-dependent paradigms.

If we assume the critical band to be two-and-one-half times larger than the critical ratio in animals as it is in humans, then the critical bands can be estimated from the data shown in Table I. This is done by taking the antilog of the critical ratio and multiplying it by 2.5 (Scharf, 1970). Table II shows critical bandwidths estimated in this way. These estimates were to be used as a general guide for determining bandwidths in the critical-band experiment.

TABLE III. Results of the critical band experiment for group I, group II, and group I and II combined. The following data are given for each test frequency: masker bandwidth (BW) in Hz, mean masked threshold ($\bar{x}$) for each BW in dB SPL, standard deviation (SD) and approximate critical band (CB) in Hz (for combined groups only).

	Group I			Group II		Group I and II	
	BW	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0.5 kHz	710	48	7.636	54	8.308	50	8.476
Approx. CB=500	700	50	4.874	53	6.665	51	6.003
	520	49	5.582	55	7.366	52	7.217
	510	49	4.478	54	7.113	51	6.448
	490	46	6.003	49	7.949	47	7.131
	410	45	7.131	45	6.821	45	6.983
	260	42	7.949	43	5.583	43	6.903
	160	42	7.554	46	5.772	44	7.000
	BW	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1 kHz	600	59	9.473	63	6.448	61	8.333
Approx. CB=500	550	57	7.615	59	4.968	58	6.455
	515	59	8.258	64	7.796	61	8.334
	480	58	8.329	58	6.448	58	7.453
	425	52	8.425	56	4.479	54	7.022
	390	50	7.071	52	5.241	51	6.377
	280	51	5.000	53	3.716	52	4.511
	BW	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
2 kHz	1150	55	6.590	59	7.510	57	7.408
Approx. CB=800	1050	57	6.768	65	8.817	61	8.810
	650	54	7.554	56	4.157	55	6.155
	550	51	8.291	51	6.155	51	7.306
	500	55	6.503	52	5.846	53	6.442
	200	49	7.870	48	5.521	48	6.776
	BW	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
4 kHz	2100	57	10.655	60	10.539	59	10.658
Approx. CB=1500	2050	56	6.665	59	10.075	58	12.236
	1750	56	8.029	61	7.636	59	8.315
	1600	57	8.257	57	5.982	57	7.217
	1450	51	7.753	54	5.951	52	7.049
	800	49	8.259	53	3.716	51	6.653
	500	48	7.265	51	5.826	50	6.730
	350	46	8.291	47	7.179	46	7.786

III. CRITICAL BAND EXPERIMENT

A. Apparatus

The electronic equipment and setup were similar to that used in the critical-ratio experiment, except the noise was filtered before being added to the signal. The

TABLE II. Mean critical ratios (in decibels) and estimated critical bands (in kilohertz).

	Frequency in kilohertz							
	0.5		1		2		4	
	CR in dB	CB in Hz	CR in dB	CB in Hz	CR in dB	CB in Hz	CR in dB	CB in Hz
Group I ($N=3$)	16	100	18	160	24	630	29	2000
Group II ($N=3$)	17	125	21	315	25	790	33	5000
Group I and II ($N=6$)	16	100	19	200	25	790	31	3150

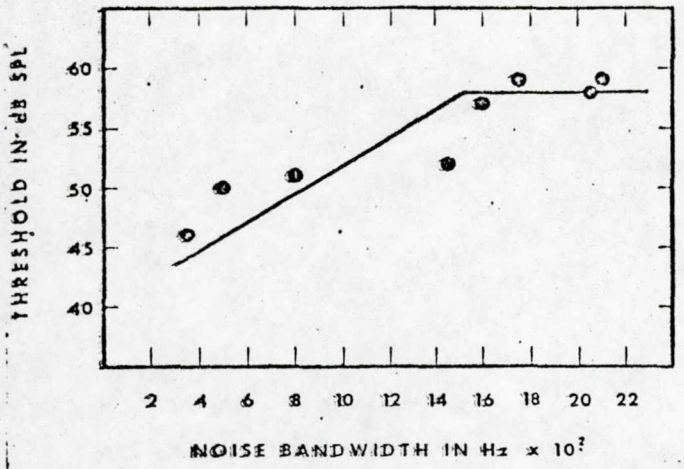

FIG. 1. Mean masked thresholds of a 4-kHz pure tone as a function of the narrow-band-noise maskers. To obtain the critical bandwidth, one line was drawn between the "unchanging" thresholds and one line was drawn through the decreasing thresholds. The intersection of the two was defined as the critical bandwidth, which, for this frequency, was approximately 1500 Hz.

experimental room for the critical-band experiment was different from the one used in the critical-ratio experiment. The sound field in this room was also measured at each of 24 positions within the experimental cage.

B. Masked thresholds in narrow band noise

All noise bandwidths used in this experiment were measured in the sound field, and the spectrum level of the noise in the band was equivalent to 25 dB SPL for all bandwidths. Thresholds were obtained for each of four test frequencies in six to eight bands of noise centered at the appropriate test frequency. Subjects were tested twice a day, seven days a week, and were tested in the same order every session. Thresholds were measured until six stable estimates were obtained for each bandwidth. There was no attempt to randomize conditions.

C. Results

The principal results are presented in Table III. Mean masked thresholds and measures of threshold variability are presented for each of the narrow-band maskers. Data for each of the four test frequencies are shown separately for each group. It should be noted that visual inspection of data plotted similarly to those shown in Fig. 1 revealed no differences in approximate critical bandwidths for the two groups. For this reason, data were averaged over both groups to compare critical ratios and critical bandwidths. Figure 1 shows a plot of the mean data for 4 kHz for the combined groups. The mean thresholds are plotted as a function of noise bandwidth, and the lines through the data points were fitted by eye.

Note in Table III that the critical bandwidth increases as a function of frequency for frequencies greater than or equal to 1 kHz. Thus, the data obtained by measuring masked thresholds in various bandwidths of noise agree with data obtained on human subjects in many experiments designed to measure critical bands. To emphasize this comparison, our data are plotted in Fig. 2 (filled circles) along with data reported by Scharf (1970, p. 161). It should be pointed out that, although the chinchilla data points appear to be parallel to those obtained in humans in the frequency range from 0.5 to 4 kHz, this may not be the case at frequencies about 4 kHz. More data above 4 kHz are necessary to evaluate the form of the function in the high-frequency region. However, it seems probable that standing-wave patterns in a sound field at high frequencies would complicate those measures.

IV. GENERAL DISCUSSION

One purpose of this study was to investigate the relations between the critical ratio and critical band in the chinchilla. Figure 3 shows the critical ratios (groups I and II from Table I), the critical bandwidths (in decibels) as a function of frequency, and additional critical ratio measures that will be discussed later. Note that the difference between the critical ratios (filled circles) and critical bandwidths (open circles) is 11 dB at 0.5 kHz, 9 dB at 1 kHz, 4 dB at 2 kHz, and 1 dB at 4 kHz. These data do not appear to show the same relationship between the critical ratio and critical bandwidths as reported by Zwicker *et al.*, (1957), and discussed by Scharf (1970). That is, the critical ratio and critical band do not differ by a factor of 2.5 (or 4 dB) as a function of frequency. The large differences between the critical ratio and critical bandwidth at the lower frequencies were particularly surprising.

Because of this apparent discrepancy, a thorough examination of the apparatus and procedure was carried out. Careful inspection of thresholds measured in other groups of chinchillas tested in this laboratory revealed a tendency for the threshold measures to *increase* (i.e., detectability was degraded) over as many as 20 mea-

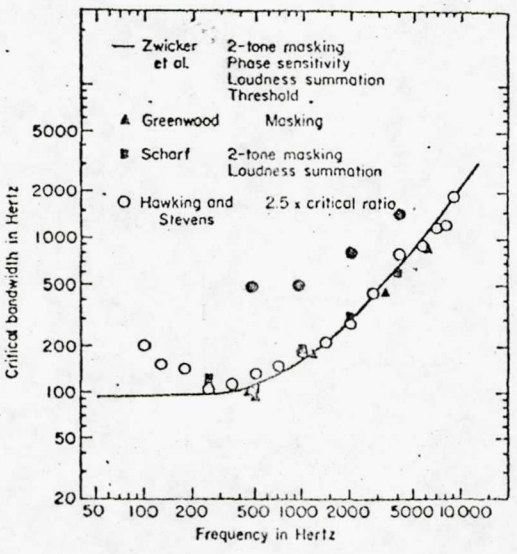

FIG. 2. The critical bandwidth as a function of frequency for chinchilla and man. The unconnected solid circles are critical bandwidths for the six chinchillas tested in this study. All the other data points were obtained from human subjects. The solid line is from Zwicker *et al.*, (1957), triangles from Greenwood (1961a) squares from Scharf (1970), and open circles from Hawkins and Stevens (1950). [Adapted from Scharf, 1970, p. 161.]

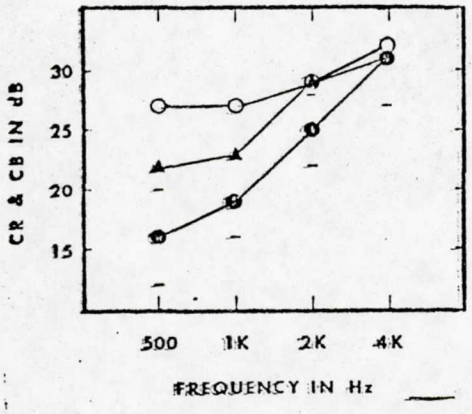

FIG. 3. Comparison of the critical ratio (CR) and critical bandwidth (CB) in decibels as a function of frequency. The data shown here are based upon means. The open circles represent critical bandwidths obtained in this study converted to decibels. The solid circles represent the mean critical ratios from Table 1 (groups I and II), while the horizontal dashes are ± one standard deviation for the data points represented by the solid circles. The triangles are the critical ratio data obtained from subjects in this study after they became well-practiced (see text).

sures at any particular frequency for any animal. The increase in threshold was approximately 5–7 dB whether measured in quiet or in noise. This phenomenon was noted in four separate groups of animals, and elimination of various hypotheses resulted in the conclusion that the degradation in performance reflected stabilization of a strict response criterion on the part of the animal. For other reasons, six more measures of critical ratios at each frequency had been obtained after many threshold measures had been taken in narrow-band noise. These data (plotted as triangles in Fig. 3) clearly indicate increases in thresholds (larger critical ratios) at 0.5, 1, and 2 kHz. Further, the latter measures are on the average within about 1 dB of those reported by Miller (1964, cited in Scharf, 1970). Therefore, it is indicated that stable measures of threshold utilizing the method of limits must be obtained from well-practiced subjects.

With these arguments in mind, the discussion will focus on the data collected from well-practiced subjects. Note that the critical ratios (triangles in Fig. 3) and critical bandwidths are not parallel functions of frequency in the chinchilla as they are in man. The direct measure of the critical bandwidth does not increase as rapidly with an increase in frequency as does the critical ratio.

At this point, no adequate explanation can be offered for these results. However, there are other data that are in line with these observations. Gourevitch (1970) measured critical bandwidths at 1 and 5 kHz in monkeys (*M. nemestrina*) using the band-narrowing procedure. If the critical-band data collected on monkeys are presented in the form used in this study (in decibels), the function relating critical ratio and critical bandwidth to frequency in the monkey is virtually identical to that found in the chinchilla. More specifically, at 1 kHz the critical ratio is 18 dB and the critical bandwidth is 22 dB, or a 4-dB difference. At 5 kHz, the critical ratio and critical bandwidth are both 31 dB. At the present time, it is not possible to explain the similarity between the two animal studies and the differences they show in relation to the human data.

There is some disagreement in the literature about whether the band-narrowing paradigm is, in fact, a valid methodological approach for defining the critical bandwidth (Bos and de Boer, 1966). These authors maintain that for narrow bandwidths the masking noise and signal are so similar that a difference limen for intensity is measured. This is because of the amplitude fluctuations that are present in narrow-band noise. These fluctuations and the tonal-like quality of the masking noise are supposed to result in critical-band measures that are highly unreliable. Bos and de Boer (1966), using human subjects, showed that masked threshold and intensity difference limen vary similarly as a function of bandwidth, particularly within the critical band. They concluded that the band-narrowing type of experiment is unsuited for measuring the critical bandwidth.

This conclusion may be unjustified, however. Bos and de Boer (1966) have criticized the results of some of the band-narrowing studies on the basis that the results of these studies do not coincide with the "generally accepted values" of the critical band, which were established by Zwicker *et al.* (1957) for humans. Actually, there are only three band-narrowing studies (all utilizing human subjects) that do not fit the "generally accepted values" (Fletcher, 1940; Schafer *et al.*, 1950; Swets *et al.*, 1962). Fletcher's (1940) data were very limited and little is known about what he actually did. Schafer *et al.* (1950) had their subjects match the tone and the noise in pitch so it is difficult to know exactly what frequency was used at any bandwidth. Swets *et al.* (1962) measured their critical bandwidths on the assumption that the internal or auditory filter was either rectangular, Gaussian, or single-tuned, and arrived at different critical-band values for each assumed internal filter.

There are also three band-narrowing studies (also utilizing human subjects) that have obtained values of critical bandwidths that are similar to the "generally accepted values" of the critical band (Hamilton, 1957; Greenwood, 1961; van den Brink, 1964). Hamilton has pointed out that the small differences in measurements resulting from different experimental methods and filter characteristics can yield large differences in critical band values—even in experiments of the same type. Swets *et al.* (1962) point out that "...it seems unlikely that all of these experiments are measuring *the* critical band, a fixed property of the auditory system that exists independent of experiments. It seems more reasonable to suppose that the parameters of the auditory system are not fixed, specifically, that they may vary from one sensory task to another..." (p. 109). And, Scharf (1970) has concluded that "...masking by narrow-band noise can provide adequate estimates of critical bandwidth as evidenced by the overall agreement of Greenwood's, Hamilton's, and van den Brink's measures with all the other measures of the critical band" (pp. 167–

We have also considered possible errors in our estimates of behaviorally measured critical bandwidths due to the resonance properties of the external ear.[4] The function relating the relative gain in sound-pressure level at the eardrum to the pressure in a free field for the chinchilla is quite similar to that found in man (von Bismarck, 1967). One could argue that critical ratio estimates could be in error because the spectrum level of the masker was specified by a free-field measure and was not the expected value at the eardrum. However, the sound-pressure levels of the sinusoidal test signals were measured in precisely the same manner as the noise spectrum levels and any gain in pressure over the relevant frequency regions could leave the signal-to-noise ratio unaffected. Obviously, one must know *the* critical bandwidth to decide whether a measure is severely inaccurate. On the other hand, one could argue that the band-narrowing technique could be contaminated because of inhomogeneous amplification of the spectral components of bands of noise. Consideration of these issues led to the conclusion that difficulties of this sort would lead to peculiarities in the plot of critical bandwidth as a function of frequency. Instead, the chinchilla data presented in Fig. 2 are quite parallel to those measured on human subjects who wore earphones. Consequently, we tentatively conclude that the band-narrowing procedure is an adequate method of obtaining critical-band measures in animal subjects.

V. SUMMARY

Experiments designed to obtain the relationship between the critical ratio and the critical bandwidth in the chinchilla constitute the major portion of this investigation. The assumption that the critical bandwidth is 2.5 times (4 dB) larger than the critical ratio was supported at frequencies of 0.5 and 1 kHz. However, the expected relation between the critical ratio and the critical bandwidth was not found at 2 and 4 kHz. At these test frequencies, the difference between the critical ratio and critical bandwidth were 0 dB at 2 kHz and 1 dB at 4 kHz. It was also shown that these results were very similar to those obtained in a study using monkeys rather than chinchillas. At this time, no explanation can be offered for the discrepancies found when comparing data obtained in human experiments to those data obtained in studies using animal subjects.

The major findings of this investigation are:

(1) Manipulation of false-alarm rate induced a 1 to 4-dB difference in critical-ratio size.

(2) The band-narrowing technique appears to be an adequate method for obtaining critical bands in a free field using monaural chinchillas as subjects.

(3) The function relating direct and indirect measures of critical bandwidth appears to depend upon unknown subject variables. Specifically, the increase in the critical ratio as a function of test frequency is greater for the chinchilla than it is for man.

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[1]Any complete investigation of this issue will probably be tedious and difficult. Both placement of criterion and variance in the placement of the criterion will affect performance in a manner that would degrade the animal's ability to detect.

[2]Measurements of detectability were also obtained in a paradigm that allowed for assessment of false-alarm rates so that sensitivity and criterion for responding could be studied independently. These data will be reported in a future article. Suffice to say that variation of the reinforcement parameter did indeed result in changes in false alarm rate.

[3]The intertrial intervals then occur with the following probabilities: 10 sec, $p = 0.13$; 15 sec, $p = 0.3$; 20 sec, $p = 0.3$; 25 sec, $p = 0.13$; 30 sec, $p = 0.08$; 35 sec, $p = 0.03$; 40 sec, $p = 0.03$ (Miller, 1970).

[4]We thank one of the reviewers for suggesting that the sound pressure transformation function should be considered when one discusses the relevant bandwidths of the physical stimuli.

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THE EFFECT OF NERVE GROWTH FACTOR (NGF) UPON AXOPLASMIC TRANSPORT IN SYMPATHETIC NEURONS OF THE MOUSE

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(Accepted January 22nd, 1974)

SUMMARY

To study a possible agent which controls axoplasmic transport, the rates of transport were examined in postganglionic sympathetic neurons in adult mice which had been treated with nerve growth factor (NGF). Rates were measured by observing the movement along the nerve of a front of radioactive material after a prior intraganglionic injection of L-[4,5-^{3}H]leucine. The faster of two rates observed in female mice increased from a control value of 1.22 $\pm$ 0.48 mm/h (N = 15) to an experimental value of 1.83 $\pm$ 0.53 mm/h (N = 16; $P < 0.005$) following the intramuscular injection of a total of 34 μg NGF/g body weight. In contrast, the same treatment caused the slower of the two rates to decrease from a control value of 2.32 $\pm$ 0.81 mm/day (N = 12) to an experimental value of 1.63 $\pm$ 0.70 mm/day (N = 12; $P < 0.05$). The rate of slow transport was also examined in male animals. Untreated male mice exhibit a lower rate of slow transport (1.08 $\pm$ 0.38 mm/day; N = 9) than do untreated females of equal age ($P < 0.001$). The result that nerve growth factor increases the rate of the faster phase of transport, and decreases the rate of the slower phase, indicates that this protein exerts a selective effect on transport.

To obtain an independent measure of the influence of NGF, the extent of incorporation of L-[4,5-^{3}H]leucine into tissue proteins of excised stellate ganglia was assessed after 1 h in organ culture. Animals treated with NGF showed increased incorporation of tritiated leucine. Incorporation of radioactive leucine was correlated with the rate of transport in both control animals ($r = 0.61$) and animals treated with NGF ($r = 0.67$). Within the framework of normal biological function, NGF may exert a controlling influence on both phases of axoplasmic transport in sympathetic postganglionic neurons.

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It is with great pleasure that the authors thank Dr. Harlow W. Ades for advice and counsel during the course of this research.

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EIGHTH INTERNATIONAL CONGRESS ON ACOUSTICS, LONDON 1974

ANATOMICAL ANALYSIS OF EFFECTS OF LONG-TERM, MODERATE INTENSITY NOISE ON THE CHINCHILLA

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Kokko-Cunningham A
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INTRODUCTION

Anatomical analysis of the effects of wide-band white noise presented at intensity levels of 90, 95 and 100 dB was investigated utilizing chinchillas exposed to the noise over a time period ranging from one to ten weeks. Noise exposure at these levels causes changes in the normal structure and pattern of the organ of Corti. The changes are evaluated using the surface preparation technique as reviewed by Engström, Ades and Andersson (1) and compared on the basis of intensity and length of exposure.

EXPERIMENTAL

Wide band white noise ranging from 100 to 100,000 Hz with a 3 dB per octave rise and a 12 dB per octave fall was utilized in a free-field exposure within a non-sound-absorbing room. The animals were allowed to move freely in individual cages mounted on a portable rack. Daily rotation of cages was performed to avoid positional variables. Ten chinchillas were exposed to each of the three intensity level conditions. Each daily exposure was continuous for eight hours with a sixteen hour non-stimulus interval. At weekly intervals, one animal was removed from the exposure and thus a range of damage criteria was established over the period of one to ten weeks. After a sixty day post-stimulus recovery period the animals were sacrificed and their ears analyzed for hair cell damage.

ANALYSIS

Evaluation of hair cell damage throughout the entire organ of Corti for each of 60 animal ears was accomplished by direct analysis utilizing light microscopy. Data for each ear was compiled in a computer program which produced a plot in the form of a cochleogram. Both intensity and exposure duration variables were compared on the cochleograms. Damage was quantitated by assigning each hair cell to a 1-7 scale corresponding to various morphological-histological parameters.

In addition to the foregoing, there are five graduate students who are currently in the process of obtaining their Ph.D. in this laboratory. Their degrees will be conferred in 1975 and 1976. They are:

Patricia P. McDowell: "An Evaluation of the Determination of Audiograms on the Chinchilla by Means of the Auditory Evoked Response."

Kenneth McDowell, M.S.: "Medium Duration, Low Intensity, Wideband White Noise Exposures to the Chinchilla: A study of Anatomical Damage to the Organ of Corti and Electrophysiological Measures of PTS and TTS."

Charles H. Ludmer, M.S.: "The Effects of Salicylate and Kanamycin on Hearing and Cochlear Structure in the Monkey (Macaca mulatta.)" (Beginning Fall 1974, Mr. Ludmer has been a medical student at Northwestern University Medical School, Chicago.)

David Gilchrist: "The Innervation of the Normal and Noise Damaged Chinchilla Inner Hair Cell as Studied by Serial Electron Micrographs."

William H. Seaton, M.S.:[27] "Critical Bands in Impaired Ears."

There have been students through the years (too numerous to mention) who have worked on special projects using this laboratory, thus adding more interested individuals to the field of endeavor, and who will ultimately participate in education and/or public service.

DIE MORPHOLOGIE DES GANGLION SPIRALE COCHLEAE

VON

BERNHARD KELLERHALS[1]

unter Mitwirkung von

H. ENGSTRÖM[2] und H. W. ADES[3]

This paper describes a study of the morphology of normal and pathologically altered spiral ganglion cells. Embryology, anatomy and histology of the spiral ganglion are reviewed, and the ultrastructure of the spiral ganglion cells is discussed in detail. Special attention is given to the satellite cell sheath and to the various inclusion bodies. Although only a few species have been studied so far, two different ganglion cell types have been distinguished in several animals with respect to cell size, myelin sheath type and character of perikaryon. Unfortunately, the two cell types are not alike from one species to the other. Only in the guinea pig is the difference between the two cell types so distinct as to provide a basis for experimental work. In this species the unmyelinated cells comprise 10% of the total cell population. They are smaller in size than the myelinated cells, and typically display a fine, filamentous perikaryal structure. They are easily recognized in phase contrast sections.

The pathology of the peripheral cochlear neurons following damage to the end organ has been studied in guinea pigs. In animals exposed to impulse noise and in animals injected with kanamycin, the extent of the damage to the organ of Corti has been assessed by the surface specimen technique and has been compared with damage in corresponding spiral ganglion areas as seen in phase contrast sections and by electron microscopy.

Animals exposed to the damaging effect of gun-shots showed heavy damage to the organ of Corti, followed by retrograde degeneration of the peripheral neurons, proceeding centrally to involve the spiral ganglion cells and eventually the nerve fibres of the acoustic nerve.

In kanamycin-injected animals, the process of degeneration is progressive over a considerable period. The damage to the spiral ganglion is maximal in the apical region. This drug appears to have a direct toxic effect on the spiral ganglion cells as well as on the hair cells of the organ of Corti.

In regions where all outer hair cells, but no inner hair cells have been destroyed, there is usually a negligible loss of neurons in the corresponding area of the spiral ganglion, however, in a few specimens, severe damage was noted among the myelinated nerve fibers of the spiral osseous lamina, even though no loss of inner hair cells was seen.

In no instance did the percentage of lost unmyelintated cells significantly exceed the percentage of degenerated myelinated cells. They appear not to be particularly related to the outer hair cells. It can be concluded that their behaviour following noxious stimuli is no different from that of their myelinated counterpart.

The process of degeneration as it affects the cells of the spiral ganglion, is described and illustrated. It is compared with the "primare Reizung", as it is observed in spinal ganglion cells after lesions to their axons or dendrites.

The question as to why certain ganglion cells survive while others die, remains unsolved. It is not known what changes in the organ of Corti "trigger" the eventual degeneration of the associated neurons.

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NORMAL STRUCTURE OF THE ORGAN OF CORTI AND THE EFFECT OF NOISE-INDUCED COCHLEAR DAMAGE

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CONCLUSION

Scanning electron microscopy has already added much information to our knowledge of the normal and the pathologically altered inner ear. We are at present preparing an extensive review of the use of this technique in the study of the labyrinth (Ades, Bredberg and Engström, 1970). Ades' group at the University of Illinois has developed methods for data-processing the damage seen in the organ of Corti of experimental animals. In this way, it is possible to study in detail the degeneration of all cells in the organ of Corti of an animal and to feed this information into a computer for further evaluation, and thus to create degeneration curves for comparison with other animals. The use of several techniques is necessary for an understanding of cochlear morphology.

SUMMARY

A general survey is given of the normal structure of the organ of Corti, as revealed by phase-contrast and electron microscopy and in particular by the use of the scanning electron microscope. The ultrastructure of the sensory cells is described in detail. The destructive effects on the hair cells of exposure of guinea pigs to noise of various types is discussed.

Acknowledgements

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THE SENSORY HAIRS AND THE TECTORIAL MEMBRANE IN THE DEVELOPMENT OF THE CAT'S ORGAN OF CORTI

A Scanning Electron Microscopic Study

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Abstract. The surface topography of the organ of Corti has been studied by scanning electron microscopy. Newborn kittens of various ages as well as adult cats were included in this study. In the newborn kitten all the sensory cells have one kinocilium in addition to the bundle of stereocilia. This kinocilium most frequently is short and looks rudimentary; however, some of the kinocilia are even longer than the longest of the stereocilia. The distal end often shows a bulb-like thickening. Within a few weeks after birth, all the kinocilia disappear. The relationship between the outer margin of the tectorial membrane and the epithelial surface has been investigated. In the kitten the tectorial membrane is firmly attached at its outer margin to the upper, free surfaces of the outermost row of Deiters' cells. There is no such attachment in the cat six weeks of age nor in the adult cat. This may have functional implications.

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COMPARISON OF HEARING THRESHOLDS AND MORPHOLOGICAL CHANGES IN THE CHINCHILLA AFTER EXPOSURE TO 4 kHz TONES

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(Received January 18, 1974)

Abstract. Behavioral, anatomical and histochemical effects of acoustic overstimulation produced by a 4 kHz tone were investigated. In each of five monaural chinchillas, high frequency hearing loss was produced which was related to locus and extent of hair cell loss. Masked audiograms were fairly consistent with those obtained in quiet and the anatomical data on presence or absence of hair cells. Histochemical and electron microscope examination of selected portions of the cochlear partition revealed a series of changes in hair cells which may or may not influence their functional properties.

Tones or noise of high intensity have been used for many years to stimulate the ears of experimental animals and thus to produce damage to ear structures and changes in function of the inner ear. Periodically, development of new techniques for examining inner ear pathology and for measuring inner ear function has permitted the closer and closer correlation of the physical characteristics of the stimulus, inner ear pathology, and inner ear function.

The best of the older studies employed electrical responses by inner ear elements or behavioral methods to obtain measures of auditory acuity. They related inner ear function with the familiar parameters of acoustic input; frequency, intensity, and duration of sounds used as stimuli. However, in very few of these studies have ear damage and ear function been studied in the same animal after controlled exposure to sound.

The present study attempts to correlate these three components as well as is possible with presently available equipment and techniques. All of these are inevitably fallible. The "thresholds" obtained from the conditioning method used are simply the levels at which the animals stopped responding. Whether or not they *could* have responded at lower levels of sound is open to unanswerable conjecture. The defense of the method is the consistency with which they did respond, and the differential effects with respect to frequency of stimulus upon exposure to sound.

The anatomical methods are the best available at present. Improvements have come through the use of the surface preparation, aided by examination of selected portions by transmission electron microscopy and use of scanning electron microscopy to survey larger areas. Each of these methods has, at its best use, certain shortcomings. These can be minimized, but not eliminated, by the judicious use of all the methods in combination. Anatomical techniques, however refined, still have the unfortunate characteristic that they are methods of looking at artefacts. It is true that we have reduced the artefacts to fairly manageable proportions and are finding features not hitherto available, but this raises more questions than are answered.

The methods of producing sounds and exposing the animals to them are perhaps less questionable than the others. The frequency is well controlled, and the levels, while high, have only moderate distortion. That is, the harmonic and sub-harmonic frequencies are not high enough in pressure to produce more than negligible damage at worst for the relatively short times

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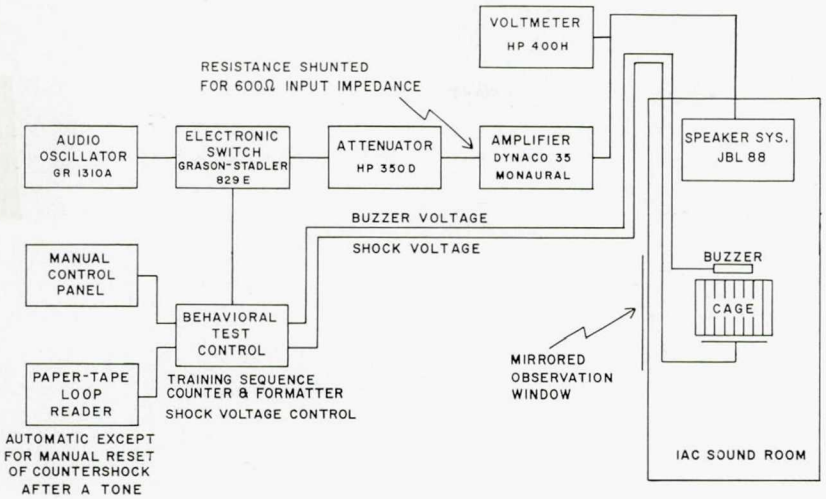


Fig. 1. Diagram of testing apparatus.

of exposure. The exposure itself is more open to question. We have tried putting the sound directly to the tympanic membrane through a speculum. This makes it possible to measure the intensity of the stimulus with a probe tube from the microphone inside the orifice of the speculum. A discrepancy may arise in that the animal, even when anesthetized and clamped as securely as possible, may still achieve slight movements. Even a slight movement may move the speculum to a quite different position and so a quite different intensity of sound may be delivered to the tympanic membrane. We have also tried delivering the sound from a speaker three inches from the pinna, and aimed directly at the external orifice of the external auditory meatus, with the probe tube just inside the orifice. This gives a constant sound level, but we do not know precisely what the sound level is at the drum membrane. This seems to be the most consistent way we can deliver the stimulus; therefore, despite the doubt as to what the level is at the tympanic membrane, it seems better to achieve the consistency where it can be measured accurately.

MATERIALS AND METHODS

This study was conducted along classical lines. That is, the work can be divided into the following separate parts: Behavioral training and determination of audiograms; exposure to pure tones; redetermination of the audiograms; sacrifice, and examination of the organ of Corti. These represent distinctly separate disciplines which should be managed by experts in each area, thus the multiple authorship of this paper. Dr Ades was in overall charge of the project and also participated especially in the anatomical work; Dr Kokko-Cunningham took part in the anatomical portion and had the special responsibility for the electron microscopy and histochemistry; Dr Trahiotis supervised the animal testing portion; and Dr Averbuch, who is an electrical engineer, supervised the exposure and the instrumentation.

PROCEDURES

Apparatus

Five monaural chinchillas were trained in the apparatus which is diagrammed in Fig. 1.

The output from the audio oscillator was delivered to the electronic switch for gating the tone on and off. The gated tone was adjusted in amplitude with an attenuator from a known value of speaker drive (measurements taken with a voltmeter for a continuous tone).

The behavioral test apparatus, which was built in this laboratory, contained the electronics necessary to program the sequences of stimuli and to operate the buzzer and shock device. The

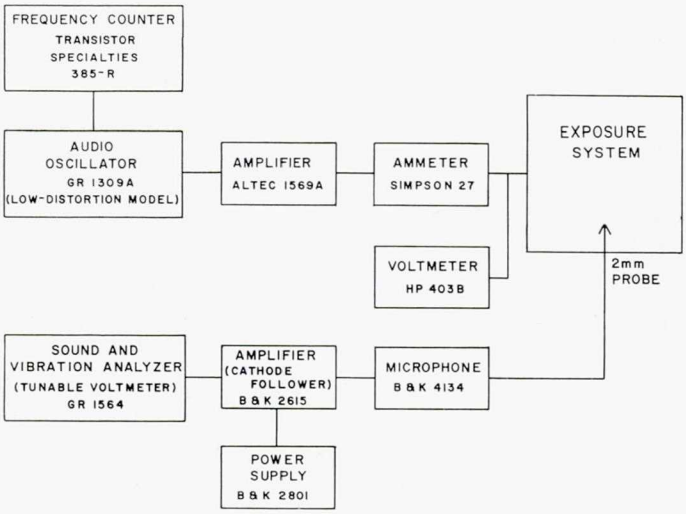


Fig. 2. Diagram of exposure apparatus.

training sequences could be manually triggered from the control panel, or could be sequenced automatically by a paper-tape loop. The paper tapes were computer generated and provided the desired randomization of intertrial interval.

The sound pressure level (SPL) for a known value of speaker input voltage at one frequency was measured for all frequencies at 24 points in the training cage. The reference SPL used at any frequency for threshold determinations was the average of these 24 readings. Calibration in this manner allowed all SPL's at all frequencies to be known with respect to a single, pre-set drive setting without the need for readjustment.

Audiometry

The chinchillas were trained to avoid shock by crossing a barrier in the shuttle box (see Fig. 1) in response to presentation of a 2-sec sample of a sinusoidal tone of 1000 Hz. Shock was accompanied by sound from a buzzer mounted in the roof of the cage. Thus, the sound of the buzzer acquired aversive value and could substitute for the shocks to keep the animals motivated in the months of future testing.

When the animals had reached a criterion of not less than 90% correct avoidance responses, the stimulus parameters were changed, and threshold determinations were begun using a method of descending limits. The duration of test tones was 750 msec with a rise/decay time of 50 msec. The stimulus consisted of three tone bursts separated by 250 msec. If the animal moved from one end to the other of the shuttle box at any time during the presentation of the three tone bursts, shock was not delivered and a correct response was recorded. The SPL of the stimulus was then attenuated by 20 dB and the test was repeated after a randomly chosen time interval (averaging about 20 sec). This pattern was repeated until the animal no longer responded. The SPL was then increased 10 dB. If the animal responded correctly, the SPL was reduced 10 dB and the sequence continued. If the animal *failed* to respond to the 10 dB increase in SPL, the procedure was discontinued and the threshold was taken to be midway between the latter level and the level which produced the last positive response. Each animal was tested at 2, 4, 8, and 16 kHz, *or* 0.125, 0.250, 0.500 and 1.0 kHz each day. Since the animals got better and better at detecting low level stimuli, many measurements of "threshold" had to be taken. When these remained relatively stable over at least 20 sessions at each frequency, a threshold value was calculated.

Following completion of the tests in quiet, the animals were tested at three levels of masking using a low pass noise filtered at 10 kHz that was presented continuously at nominal spectrum

levels of 0 dB, 10 dB, and 20 dB SPL. Measurements of levels of noise were made at 24 positions in the test cage. The sound field was quite uniform. Each animal was tested at least eight times at each test frequency for each of the three levels of masking noise.

Exposure to pure tones

Each of the animals was anesthetized and exposed to presentations of a 4 kHz tone for one hour at 130 dB, 125 dB, or 120 dB SPL. A block diagram of the apparatus is shown in Fig. 2.

A specially constructed plastic chamber was used for exposing chinchillas to sound levels up to 140–145 dB SPL. The maximum attainable levels were dependent upon frequency, due to resonances in the rectangular exposure chamber. The basic chamber was made of one-inch thick transparent plastic, 4 inches high, 4 inches wide, and 13 inches long internally and was open at the ends. On one end, any of a variety of speaker fittings could be attached. A single Altec 421A, 15-inch, low-frequency speaker could be mounted, with an adapter, for frequencies below 500 Hz. For the lower frequencies, heavy aluminum extensions could be used to tune the length of the chamber to the desired resonant frequency while the end of the chamber, opposite the speaker, was sealed with an aluminum plate. For higher frequencies, a pair of Altec 290 D speaker drivers were connected to the chamber through a cast-aluminum horn and were driven in phase. When necessary to reduce chamber resonance, an acoustic absorbing chamber was attached on the end of the exposure chamber opposite the speaker.

With the animal's head in the chamber, a 2 mm probe was inserted into the ear of interest, and was brought as near as possible to the ear canal opening. The probe, attached and calibrated to a one-half inch microphone, was left in place during the entire exposure. In addition to the SPL of the principle exposure frequency, the first few harmonics and subharmonics were checked with a tunable voltmeter to insure against excessive distortion. The absolute sound-level calibration was made with a B&K 4220 "Pistonphone" coupled to the one-half inch microphone. The probe was calibrated, over the frequency range of interest, against a second one-half inch microphone.

Anatomical investigation

The surface preparation used for the initial phases of anatomical study was described in Stockwell et al. (1969). It will not be repeated here. The following histochemical methods were employed.

The cochleas (with the exception of animal No. 89) were fixed with 2 % glutaraldehyde in 0.1 M cacodylate buffer of pH 7.2. The fixation was begun by diffusing the fixative gently through the fenestra ovalis and the fenestra rotundum, with a small outlet hole made in the apex. It was continued by immersing in the same fixative for 6 hours. During the latter period the bony walls of the cochlea were ground down and the coils of the organ of Corti were detached and removed from the modiolus. The resultant segments were treated as free-floating pieces thereafter. When fixation had been completed, the segments were rinsed from 12 to 24 hours in 0.1 M cacodylate buffer (pH 7.2) which contained 0.2 M sucrose. They were then incubated for acid phosphatase activity as described by the Barka-Anderson (1962) modification of the Gomori reaction (1952). Incubation time was 60 min at 37°C, after which the segments of the organ of Corti were rinsed again and then placed in 1 % OsO_4 in cacodylate buffer for 1 hour. Following another rinse in the buffer, the segments were mounted in glycerine under cover glasses. They were then ready to be observed under the light microscope. Animal No. 89 was fixed directly in 1 % OsO_4 buffered with veronal acetate and no incubation was done.

After recording the presence, absence, or damage of hair cells of the organ of Corti (the resultant computer-drawn cochleogram will be illustrated for each animal), the segments were removed from the slides, dehydrated, embedded in Epon–Araldite, and tissue from representative areas was thin-sectioned. In some specimens, only a narrow area was sampled for elec-

tron microscopy, while in others segments up to 1000 μm in length were sampled at 25 to 50 μm intervals. Thin sections were stained with uranyl acetate and lead citrate and viewed in either the RCA EMU3H or the Siemens Elmiskop A1.

RESULTS

Pre-exposure data

The mean thresholds of the 5 animals taken in quiet circumstances are shown in Fig. 3 in solid line. In addition, the similar curve for Miller's 36 animals is shown in broken line (Miller, 1970). This demonstrates a comparison with the results of at least one other investigator, and shows general agreement with work done on the same species at a different laboratory, with different apparatus. The two differ significantly only at the highest frequencies, 8 kHz and 16 kHz. This may be the result of some slight difference in the test chambers which is impossible to analyze.

An indication of the variability of threshold measurements can be gained from Fig. 4, in which representative data from 2 animals, Numbers 90 and 96, are plotted as a function of number of thresholds included in the determination of average threshold. Standard deviation was fairly constant at about 5–10 dB in the last five measurements. Fig. 5 shows the average thresholds of the 5 animals for four conditions of pre-exposure testing, i.e., quiet, and 0, 10, and 20 dB levels of masking. No data were collected at 16 kHz in the presence of masking noise because of the variability in the sound field, and the non-uniform speaker response which precluded adequate definition of the level. Note that the thresholds were systematically affected by the level of the masking. The apparent decrease in sensitivity of the animals at 1 kHz is due to the fact that the sound system had a resonance point at that frequency.

It is possible to compare the signal level to masker level ratios necessary for detection at each test frequency to the data reported by Scharf (1970, p. 176) (from Miller, J., unpublished observations). In order to make these comparisons,

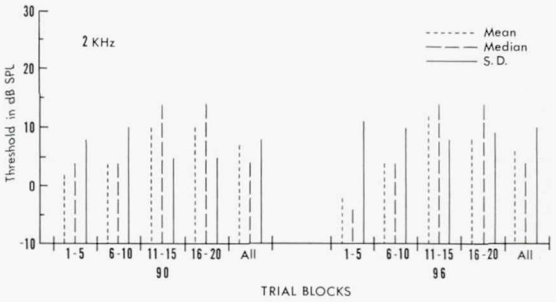


Fig. 4. Samples of mean, median, and standard deviation of pre-exposure threshold responses of animals No. 90 and No. 96 in blocks of five trials.

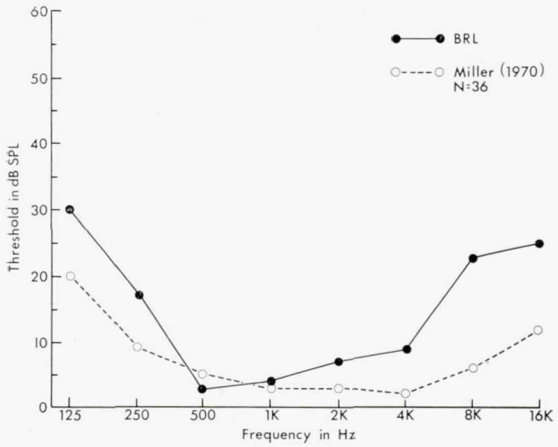


Fig. 3. Average pre-exposure thresholds of the 5 animals included in this study (——), and of Miller's 36 animals done at Central Institute for the Deaf (----).

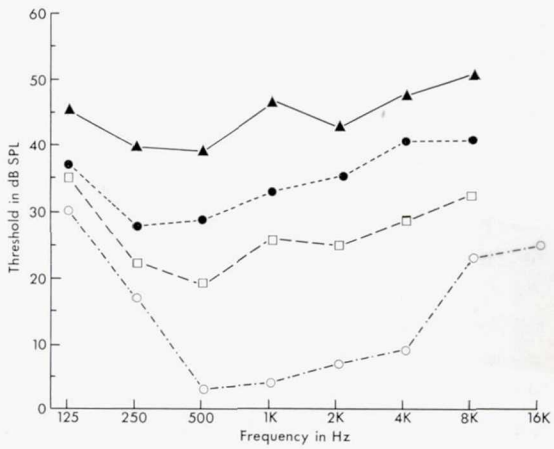


Fig. 5. Pre-exposure thresholds in quiet (○), and at the three levels of masking (□ = 0 dB level, ● = 10 dB level, ▲ = 20 dB level).

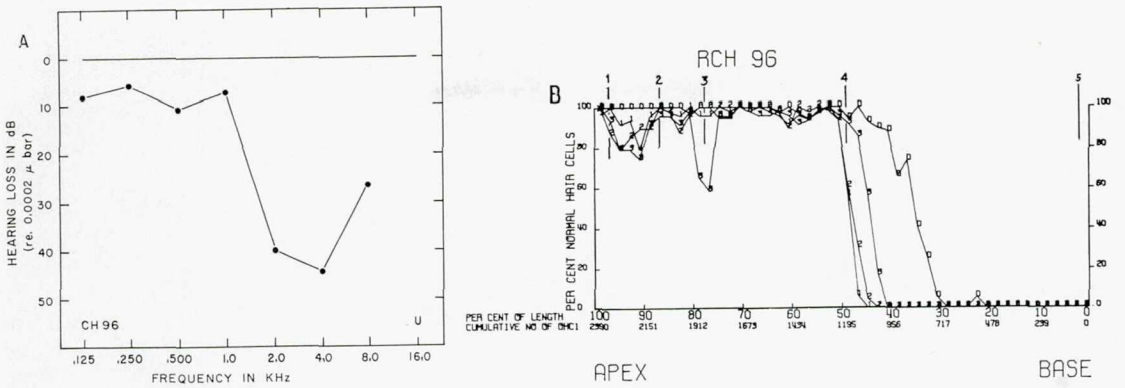


Fig. 6. (A) Hearing loss, animal No. 96, following exposure to 4 kHz, 130 dB, for 1 hour. (B) Cochleogram of animal No. 96 (0 line = inner hair cells, 1, 2, 3 lines = rows of outer hair cells from modiolar side out). Vertical lines and numbers indicating where samples were taken for EM.

the actual SPL value of the noise spectrum level measured in the test cage was utilized. The signal to spectrum level ratios measured in the two studies typically do not differ by more than 2 dB for the test frequencies used in both studies. This result is interpreted as further evidence of the adequacy of the sample and the testing procedures.

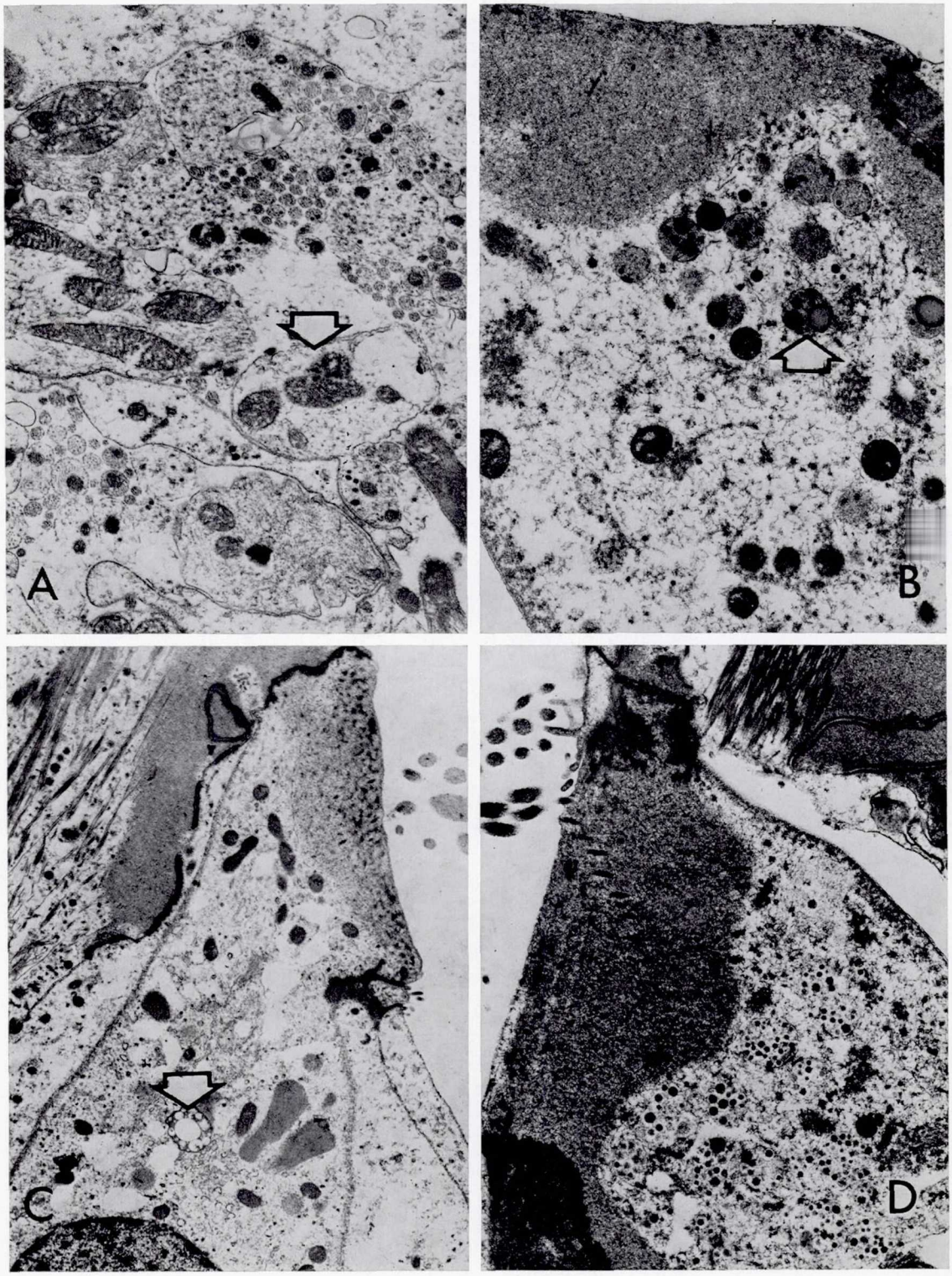
Post exposure data

Animal No. 96. Fig. 6 is a composite of the data on this animal. Fig. 6A is the curve of hearing loss with normal hearing assumed to be a straight line, as is ordinarily done with human data in an audiogram. Loss of hearing for the several frequencies is represented as points below the straight line. There is negligible hearing loss evident at the first four frequencies; however, there are losses of 40 dB at 2 kHz, 44 dB at 4 kHz, and 27 dB at 8 kHz. Ostensibly, the loss peaks at 4 kHz, but it will be remembered that the standard deviation is roughly 5 dB, so a 10 dB loss is probably significant, but must be considered as marginally so.

The computer-drawn cochleogram (Fig. 6B) shows complete cell destruction from the base up to 30 % of the length, rising sharply from that point indicating a virtually intact cochlea at 50 % of the length. The inner hair cells appear first, about 75 % of them being present at 35 % of the length, and 90 % or higher from 40 to 50 % of the length. There is little difference between the rows of outer hair cells in this respect. There is scattered cell loss at the apical end with only the third row of outer hair cells showing appreciable loss. Samples were taken from the specimen for electron microscopy from five areas. The areas are marked by vertical lines in Fig. 6B.

In the first three areas, in addition to the missing cells, other changes that were considered abnormal were seen. Some fibers in the inner spiral bundle and nerve endings underneath the inner hair cells appeared swollen (Fig. 7A). Outer hair cells in all three areas showed an accumulation of inclusion or multivesicular bodies under the cuticular plate (Fig. 7B). These vesicles were surrounded by membranes and contained granular or fibrillar material and a few lipid droplets. They varied in size from 0.5 to 2 μm. The inclusion bodies occurred in moderately increased number as compared with the normal animals, and some of them exhibited acid phosphatase activity. Lamellar structures, somewhat resembling tightly packed lateral membranes were also seen under the cuticular plate in the outer hair cells of these areas.

In area 4 many more of the multivesicular bodies appeared in the outer hair cells; however, the region in which the cells contained abnormal numbers of these was relatively narrow, extend-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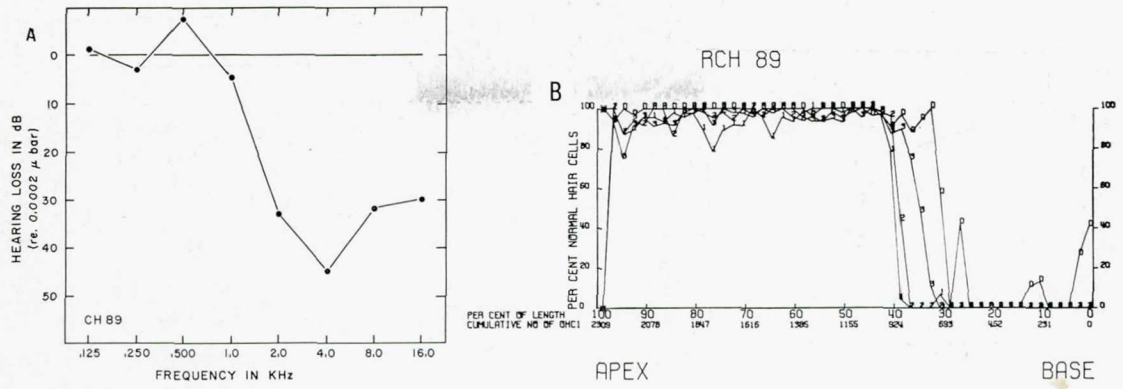

Fig. 8. (A) Hearing loss, animal No. 89, following exposure to 4 kHz, 125 dB, for 1 hour. (B) Cochleogram of animal No. 89 (see legend for Fig. 6B).

ing only 200 μm from the area of heavy damage (this is in contrast to animal No. 93 (see below) which had a wider area of abnormal outer hair cells near the edge of heavy damage). In addition, nerve fibers of the inner spiral bundle were swollen, and occasionally fused, or giant stereocilia on both inner and outer hair cells were apparent. Rods or crystalloids exhibiting a tubular substructure were more numerous than those usually seen in inner hair cells of normal animals and showed signs of degeneration in that they contained occasional lipid droplets (Fig. 7C), and sometimes showed acid phosphatase activity.

Area 5, 400 μm from the base of the cochlea, consisted only of a flat epithelium. Cytoplasmic organelles were scarce. The cells of the limbus spiralis were fewer than normal and quite shrunken while the Böttcher cells appeared quite normal.

Animal No. 89. This animal showed a hearing loss curve (Fig. 8A) much like the preceding one with loss peaking at 4 kHz. The peak is at -45 dB. The only other frequencies which show significant losses are 2, 8, and 16 kHz.

The cochleogram (Fig. 8B) shows essentially a complete absence of hair cells (except for a minor number of inner hair cells) up to 30% from the basal end of the organ of Corti. This is a fairly sharp-edged lesion with the inner hair cells again virtually completely present within 2%, i.e., at about 32% of the length. The outer hair cells are all present once more at about 43% of the length. From that point on to the apex there is scattered, minor loss of outer cells and the inners remain at full count.

Electron microscopy was done, but the ultrastructural preservation was inferior to the other 4 animals. Consequently, about all that can be said affirmatively is that there was an abnormal accumulation of multivesiculated bodies in the outer hair cells near the major lesion. Systematic sampling was not carried out.

Animal No. 97. This animal showed distinct loss of hearing at 0.250, 0.500, and 1.0 kHz, and a much greater loss at 2, 4, and 8 kHz; the

Fig. 7. (A) Inner spiral bundle from area 1 (middle of apical coil) of animal No. 97. Some of the nerve fibers are swollen (*arrow*) and have empty spaces. Others are swollen and filled with fibrillar material. $\times$ 11 000. (B) Outer hair cell 2 from area 4 (end of middle coil) of animal No. 93 showing accumulation of multivesicular inclusion bodies (arrow points to one) under the cuticular plate. Inclusion bodies are surrounded by a membrane and contain fibrillar or granular material and lipid-like droplets. $\times$ 11 700. (C) Inner hair cell from area 4 (end of middle coil) of animal No. 96. One of the rods with tubular sub-structure shows accumulation of lipid (*arrow*). $\times$ 6 300. (D) Outer hair cell 1 from area 6 (lower basal coil) of animal No. 93. Multivesicular inclusion bodies have increased in number and fill the subcuticular cytoplasm. Some have lost their surrounding membranes and small lipid-like droplets are dispersed in the cytoplasm. $\times$ 11 200.

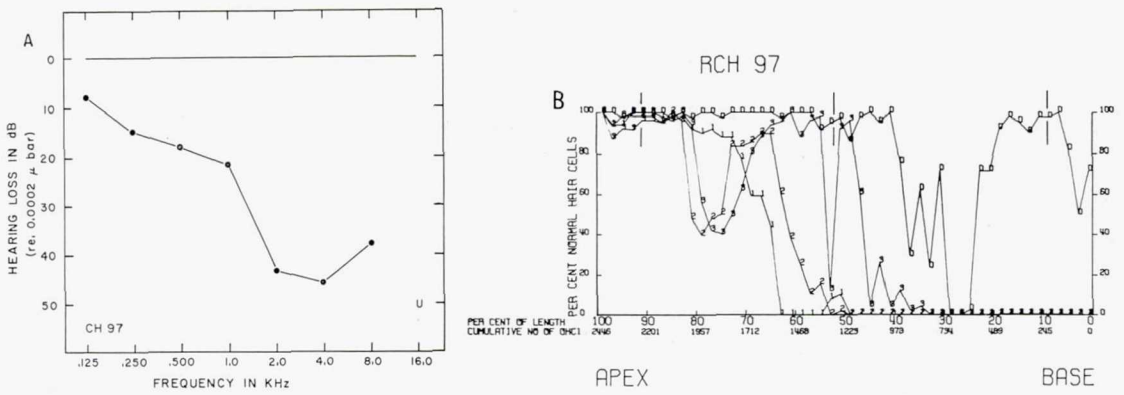

Fig. 9. (A) Hearing loss, animal No. 97 following exposure to 4 kHz, 130 dB, for 1 hour. (B) Cochleogram of animal No. 97 (see legend for Fig. 6B).

animal did not respond to 16 kHz at the highest SPL which could be offered at that frequency (Fig. 9A). It was a less distinctly peaked loss than the preceding two (Nos. 96 and 89).

The cochleogram (Fig. 9B) shows a much greater loss of cells than in animals Nos. 96 and 89. The inner hair cells are irregularly absent for the first 40 % from the base, and essentially present for the rest of the way to the apex. The outer hair cells are virtually all destroyed for nearly 50 % of the distance from the base. Thereafter they are moderately to severely impaired up to 81 or 82 % of the distance from the base, while for the remaining 18–19 %, they appear to be essentially intact.

Three samples were taken from this specimen for electron microscopy. They are indicated by vertical lines drawn onto the cochleogram (Fig. 9B). In the first specimen (counting from the apex) the hair cells looked fairly normal, although occasional giant hairs were seen on inner hair cells. Numerous swollen nerve endings and fibers were seen under the inner hair cells and in the inner spiral bundle (Fig. 7A). Some of the endings appeared to be swollen and empty and had broken membranes, while others had fibrillar material replacing the synaptic vesicles. The number of abnormal nerve endings was much higher than in animal No. 96. Occasional multivesicular bodies were seen in the outer hair cells.

There were only inner hair cells in the second area, the outers being entirely destroyed. The inner cells showed numerous degenerative rods and many giant stereocilia, and most of the nerve endings were swollen. The outer hair cells were missing from this area, but the Deiters' cells and pillars were present and maintained the general contours of the acoustic papilla. The Deiters' cells contained huge inclusion bodies that appeared to enclose membranous material and degenerating mitochondria. The inclusion bodies probably consisted of cell debris from degenerated hair cells.

The third sample contained inner hair cells, but no outer hair cells, leaving only Deiters' cells to maintain the general contours of the outer hair cell area. The inners contained numerous degenerative rods and were also subtended by many swollen nerve endings. Deiters' cells in the outer hair cell area contained numerous acid phosphatase positive lysosomes together with acid phosphatase positive Golgi complexes. Material that could have been interpreted as cell debris was rarely encountered. The endolymphatic surfaces of the supporting cells were covered with microvilli, and there were occasional large vacuoles inside the supporting cells which were lined with similarly numerous microvilli. The Böttcher cells again seemed normal in all respects.

Animal No. 90. This animal showed slight hearing loss at 1, 2, 4, 8, and 16 kHz; and none

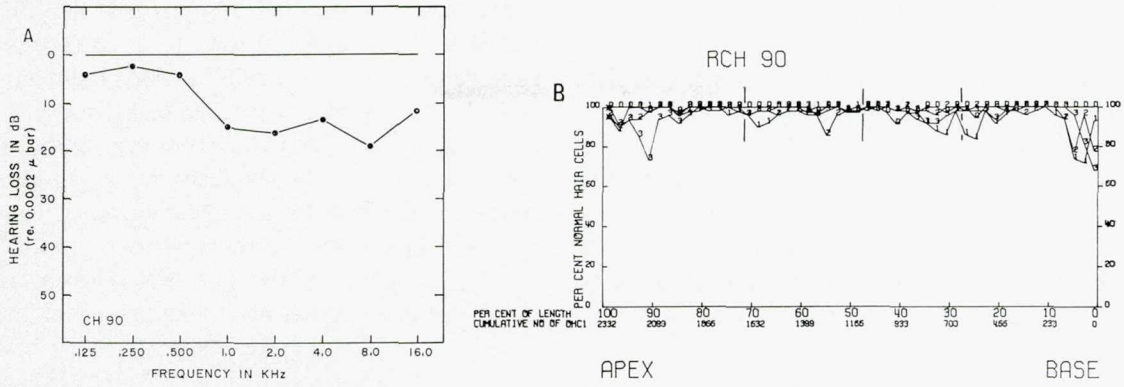

Fig. 10. (A) Hearing loss, animal No. 90, following exposure to 4 kHz, 120 dB, for 1 hour. (B) Cochleogram of animal No. 90 (see legend for Fig. 6B).

at the lower three frequencies, 0.125, 0.250, and 0.500 kHz (Fig. 10A). This statement is based on the thesis that nothing below a 10 dB loss should be counted as an actual loss.

The cochleogram (Fig. 10B) shows little damage. The greatest amount is just at the base and up to 10 % of the length, the loss being about equal in the three rows of outer hair cells. The remainder of the length shows only significant deviations of one of the rows at a time, one such deviation near the apex showing 25 % damage to the third row of outer hair cells. The inner hair cell row is intact throughout. Samples for electron microscopy were taken from the areas indicated in Fig. 10B.

Apart from the missing cells only a few ultrastructural changes were interpreted as abnormal. Outer hair cells in all areas sampled showed an accumulation of inclusion or multivesicular bodies under the cuticular plate. There were occasional giant stereocilia in all the samples, more frequently on inner than on outer hair cells. The nerve endings and supporting cells appeared normal.

Animal No. 93. This animal had apparently significant losses of hearing at 0.250 and 4 kHz, and normal hearing otherwise (Fig. 11A). The loss at 4 kHz would fit the results on other animals; at least it would not be discordant. The loss at 0.250 kHz, however, fails to match the results on the others. In no other case are there losses at two test frequencies with an intervening interval of three test frequencies at which no loss occurs. Whether this is to be "explained" on

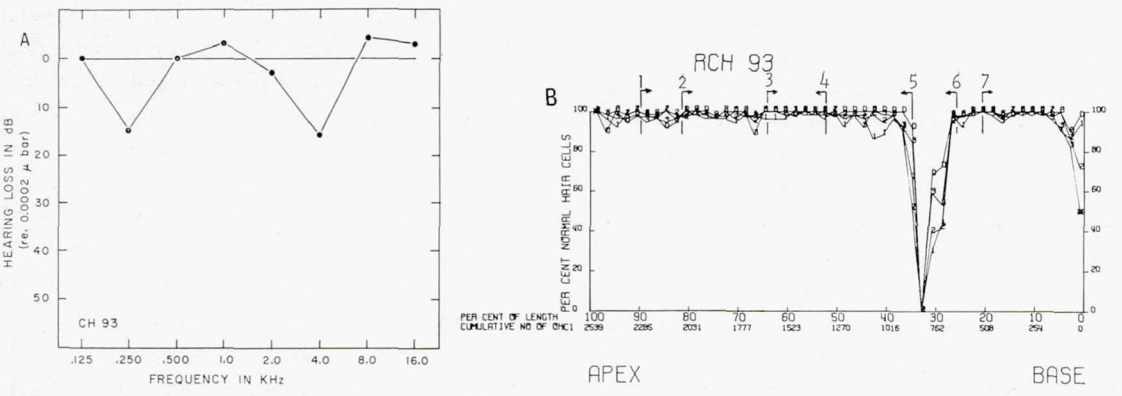

Fig. 11. (A) Hearing loss, animal No. 93, following exposure to 4 kHz, 125 dB, for 1 hour. (B) Cochleogram of animal No. 93 (see legend for Fig. 6B).

the basis of some unknown error in testing, or on the microscopic results remains to be seen. The cochleogram (Fig. 11 B) shows a complete loss of cells over a short distance at about 30–39 % of the length counting from the base. This might account for the 4 kHz loss in hearing, but there is no separate lesion to account for the loss at 0.250 kHz. The two hearing losses are of about equal magnitude. If the 4 kHz loss is to be accounted for as being all that would be expected from a narrow lesion in the basal turn, why is there no obvious lesion in the apical turn to account for the loss at 0.250 kHz? On the other hand, if the result on 0.250 kHz is to be accounted for on the basis of testing error, why is that at 4 kHz not equally spurious? The specimen was sampled at seven regions shown by vertical lines in Fig. 11 B.

The first four areas (counting from the apex) all showed swollen nerve endings under the inner hair cells and swollen fibers in the inner spiral bundle. Outer hair cells showed an increase in the number of inclusion bodies under the cuticular plate (Fig. 7 B). Rods or crystalloids exhibiting degenerative changes were seen in inner hair cells; however, we are not prepared to say that the changes noted are related to the hearing loss at 250 Hz.

In areas 5 and 6 the main lesion consisted of a central area about 250 μm in width in which the acoustic papilla was absent and was represented only by flat cuboidal epithelium which was relatively empty of cellular organelles. The hair cells were missing in an area about 250 μm wide in either direction, but in these zones the supporting cells were present, filling the space formerly occupied by hair cells, and maintaining the general contour of the organ of Corti. There were acid phosphatase positive lysosomes and Golgi complexes in the supporting cells. Cell debris from degenerated hair cells was rarely seen, and in these areas there were numerous nerve fibers which were quite normal in appearance. In the region from 750–1000 μm away from the maximum damage area, the subcuticular portions of the outer hair cells were filled with multivesicular bodies (Fig. 7 D) which contained large numbers of lipid-like droplets. In the area from 500–750 μm from maximum damage were outer hair cells in which the multivesicular bodies not only occupied the subcuticular region, but filled the entire cell. Some of the multivesicular bodies had lost their surrounding membranes, and their contents, consisting of lipid droplets, were dispersed throughout the cytoplasm of the hair cells. There were numerous degenerated nerve endings under the outer hair cells in the area also. The inner hair cells contained many tubular rods; some of them showing degenerative changes. Giant stereocilia were relatively frequent.

In area 7, the outer hair cells still had more than the normal complement of multivesicular bodies in the subcuticular region. Under the inner hair cells there were many swollen nerve endings. Inner hair cells also contained degenerative rods with occasional acid phosphatase activity. Numerous giant stereocilia were seen.

DISCUSSION

Many articles on the correlation between hearing loss and damage to parts of the inner ear start with at least an implicit assumption that the place of maximum vibration of the basilar membrane determines receptor representation of acoustic stimuli. By a curious reciprocal device this "place" theory of hearing then is used to explain the data, while the data provide evidence for a "place" theory of hearing. It is true that if one looks only at 4 kHz exposures, the agreement with a simple place theory is fairly good but by no means perfect. The present results apply only to exposures of 4 kHz and are therefore biased toward belief in a place theory. They should not be so considered, and the place theory arguments should be held in abeyance for the moment. This paper is designed to speak rather to the problem of correlation of damage to the inner ear and damage to function.

In that respect there are some apparent discrepancies between hearing results and cochlear pathology in animals Nos. 89, 96, and 97. According to a rigorous place theory, for example,

the loss at 4 kHz should be much higher than it is, if not complete. Likewise, hearing for the higher frequencies should be as much impaired as for 4 kHz if not more, inasmuch as the receptors were destroyed for the more basal portions of the cochlea as well as the presumptive 4 kHz region. In actual fact, however, a considerable amount of hearing remains for those frequencies. What receptors are transducing the higher frequencies?

Before attempting an answer to that question, let us turn to the histological methods for a moment. A source of error may be found in the manner of judging the condition of the cells from the construction of the cochleogram alone. This is the object of electron microscopical and histochemical investigation of the same specimens as are represented in the cochleograms. This will form the subject of a separate publication, but some comment is warranted here. Cells in the immediate vicinity of the lesion which is shown on the cochleograms show varying degrees of abnormality including ultrastructural and histochemical changes. It must be said that the ultrastructural preservation left much to be desired. It was originally thought necessary to mount the specimens in glycerine to count them for the construction of cochleograms. We attribute part of the inferior preservation to this. In addition, the long rinses and incubation in an acid milieu after fixation also contribute to poor preservation. Even so, the changes interpreted as pathological in the present material were not found in normal animals treated similarly.

Most of the changes which we interpreted as abnormal have been described earlier. The swelling of afferent nerve endings and fibers under the inner hair cells after acoustic trauma has been reported by Spoendlin (1971). He discussed it as a reversible change. Spoendlin (1970) reported similar swellings as a result of changes in osmotic pressure (like the fused stereocilia, then, this change is not specific to acoustic trauma but is a general reaction to more than one kind of noxious influence (Engström et al., 1970). The survival time for our animals was sufficiently long to allow for the disappearance of all reversible changes, and, unlike Spoendlin, we interpret them as permanent; however, the number of swollen endings in our specimens apparently is less than those described by Spoendlin (1971), and might be presumed to be the irreversible residue of a larger number.

Increase of lysosomes in the infracuticular region has been reported by other authors (e.g., Engström et al., 1970; Ishii et al., 1968) after both acoustic trauma and treatment with ototoxic antibiotics. Ishii et al. (1967) also reported an increase in lipofuscin pigment and acid phosphatase activity in the hair cells of elderly humans. The inclusion bodies somewhat resemble lipofuscin; however, they usually have numerous small rather than a single large lipid droplet. The disappearance of the surrounding membrane, and the release of the lipid droplets into the cytoplasm have been illustrated by Spoendlin also (1970).

With survival time of 1–2 hours, Hensen bodies have been seen to increase in number and complexity after exposure to jet-engine noise (Engström & Ades, 1960). Several Hensen bodies were encountered in the hair cells of the present material, though no more than in normal ears. The accumulation of membranous material, similar to the lateral membranes, which was particularly prominent in animal No. 96, was also described by Spoendlin (1970) after acoustic trauma.

Some of the changes are designated "degenerative." These are degenerative changes on general grounds. That is, they are the sort of changes that would indicate degeneration in any cell. But how far does degeneration have to go to render the cell non-functional, or semi-functional, or functional intermittently? What does this do to threshold measurements? No answers are available immediately. We are dealing with uncertainties and variables at both ends of the process, and no method is yet known which will resolve the difficulty.

Perhaps the thresholds obtained in the presence of masking noise *following* the creation of cochlear lesions may help to explain the small amounts of hearing loss reported. One might

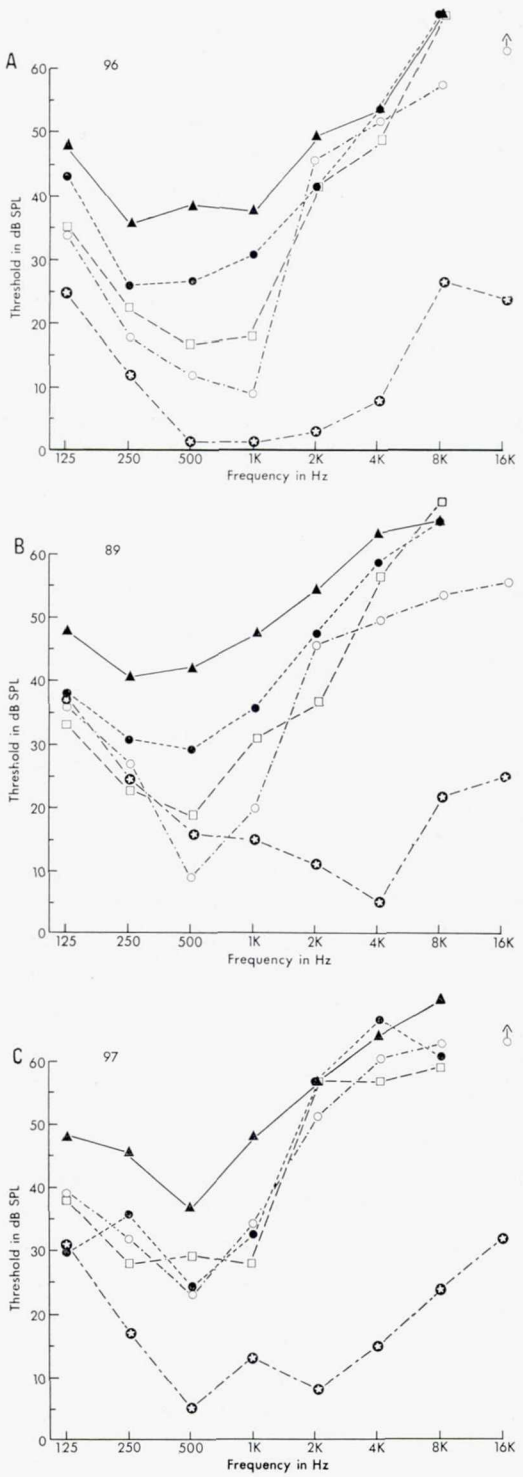

Fig. 12. (A) Animal No. 96. Curves showing thresholds obtained pre-exposure (solid circles with stars), post-exposure thresholds obtained in quiet (○), with 0 dB level masker (□), with 10 dB masker (●), and with 20 dB masker (▲). (B) Animal No. 89 (see legend for Fig. 12A). (C) Animal No. 97 (see legend for Fig. 12A).

justifiably wonder how animals 96 and 89 could, with some of the basilar membrane virtually stripped of receptors, hear so well at 8 kHz. As indicated by data in Figs. 12A and 12B, the introduction of masking noise affects performance not only at the lower frequencies, but at 8 kHz. These data may be interpreted to mean that the animals were using receptors in the *middle* regions of the cochlea to transduce the 8 kHz information. Note the presence of light microscopically healthy tissue in the relevant regions of the cochlea and the attendant masking effects (Figs. 6B and 8B).

Animal No. 97's data allow for a check on this interpretation. Note that animal No. 97 differed from 89 and No. 96 in two ways. Animal No. 97 (Fig. 9B) had various numbers of inner hair cells present in the basal region of the cochlea and a greater number of outer hair cells missing on the apical side of the lesion. One may speculate that the *inner* hair cells could be mediating the behavioral responses at 8 kHz on this animal (Fig. 12C). Further, one could predict that the masker effectiveness would be reduced at the lower test frequencies (0.250, 0.500, 1.0 kHz) because of the outer hair cell losses in the region 50–80 % of the distance from the base. Since the animal could not efficiently transduce the lower level noises, these noises could not mask. Indeed, the data indicate that this is the case. When the masker level was sufficient to mask the lower frequencies, there also appears to be a masking effect at 8 kHz. Whether this indicates that the 8 kHz sequence was processed by the animal principally in the middle portions of the cochlea by *outer* hair cells or in the basal regions by *inner* hair cells is a difficult question to answer. It seems that the data could be interpreted as indicating that the inner hair cells were transducing the information at 8 kHz and were finally masked at the highest noise level. The interpretation is based on the assumption that the outer hair cells present up to about 80 % of the distance from the base were

damaged, and, if used, would result in a greater hearing loss than that measured in the quiet.

The results on masking may actually provide support for the anatomical findings. That is to say, the remaining inner hair cells in animal No. 97, and the remaining outer hair cells in animal No. 96, may be functional in whole or in part despite the evidence of changes in them. The changes, from one point of view, may be minor and represent primarily metabolic change. This in turn would tend to vindicate the surface preparation and, hence, the cochleograms.

The results of the present experiments are at variance with those reported by Ward & Duvall (1971). There seems to be some doubt about the testing in that series of experiments (personal communication). There is also doubt that their anatomical analysis went far enough. At least we have never been convinced that positive answers were forthcoming from the type of methods that were used.

Similarly, these results have no special bearing on those of Spoendlin (1972), who reported that some 95 % of the fibers emanating from the spiral ganglion of Corti innervate inner hair cells, while the remaining 5 % innervate outer hair cells. The 5 % seems a remarkably small percentage, assuming that the outer hair cells are related functionally to loudness; however, there is no necessary reason to assume any exact relationship between loudness detection and number of neurons innervating the receptors. Perhaps the reason the percentage seems small is our preoccupation with loudness in this series of experiments.

This is not the precise, clear statement of facts one would like to present. On the one hand, the testing method is open to question. On the other, the anatomical methods are imprecise. Added to this is the suspicion that the subject of the experiments, the chinchilla, is an inadequate animal on which to base broad conclusions about human hearing which is, after all, one of the objects of research of this kind. The chinchilla is simultaneously more sensitive to noise and less sensitive to frequency differences than the human. Further studies should be done on monkeys which are apparently more closely similar to humans in those respects.

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ZUSAMMENFASSUNG

Die Wirkungen akustischer Überreizung auf anatomische, histochemische und Verhaltens-Parameter wurden an fünf einohrigen Chinchillas untersucht. Beschallung erfolgte mit einem Dauerton von 4 kHz. Die an Hand von Audiogrammen festgestellten Hörausfälle stimmten ziemlich gut mit der Lokalisation sowie der Ausdehnung des Haarzellen untergangs im Cortischen Organ überein. An Haarzellen ausserhalb der Hauptzerstörungsherde wurden ferner eine Reihe von histochemischen und ultrastrukturellen Veränderungen festgestellt, deren funktionelle Bedeutung noch unklar ist.

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