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Behavioral Ototoxicology

by W. C. Stebbins* and M. C. Rudy*

Methods for the evaluation in experimental animals of toxic substances that produce hearing impairment are described. In the experiments reported here, animals were trained by positive reinforcement operant conditioning procedures so that their hearing could be examined by behavioral means. When normal hearing was established, aminoglycosidic antibiotics (kanamycin and dihydrostreptomycin) were given daily and hearing tests administered in order that the course of hearing loss could be closely followed. Initial loss of sensitivity to the high frequencies always progressed in time to impairment at the low frequencies, and these changes in hearing were correlated with a loss of receptor cells in the inner ear which started in the basal region of the cochlea and advanced toward the apex. Although such behavioral procedures are moderately expensive to instrument and relatively time-consuming to apply, they are shown to yield valid quantitative measures of hearing. Further, they provide for reliable early detection of the toxic process and a measure of behavioral impairment that can be precisely related to the histopathological changes that occur simultaneously in the inner ear and auditory nerve.

It is our purpose in this paper to describe some of the behavioral strategies that we have found useful in studying hearing loss in experimental animals as a consequence of the chronic administration of ototoxic agents. We have employed several animal models in our research, including guinea pigs, chinchillas, cats, and both New and Old World monkeys, but here we will confine ourselves primarily to our work with Old World Monkey genera *Macaca* and *Erythrocebus*. Nevertheless, these behavioral techniques are applicable to almost any mammal that has been adapted for laboratory experimentation, and perhaps also to many that have not been so used in the past.

Intense sound, as well as a variety of therapeutic drugs, such as the salicylates, quinine, and the aminoglycosidic antibiotics, are known to produce temporary or permanent hearing loss in man and other animals. Since this paper is not intended as a comprehensive review of experimental ototoxicity but rather as an illustration of the effective behavioral procedures which are employed to study ototoxicity in experimental animals, we will focus on our experiments on the chronic administration of two aminoglycosides—kanamycin and dihydrostreptomycin. It has been shown that behavioral evidence of hearing impairment is related to discrete and orderly changes in the sensory epithelia of the inner ear: loss of receptor cells and supporting cells and degeneration of auditory nerve fibers. However, since we are concerned here with behavioral procedures and findings, we will treat the morphological changes only cursorily; they have been treated in detail elsewhere (*1*).

It is a fond hope of behavioral toxicologists—in fact, in some even a passionate desire—to establish procedures for animals which may be applied quickly, yield early evidence of toxic effects, and yet do so in a precise and unequivocal manner. This is as it should be, for experimental behavioral toxicology will reveal its true worth when its procedures are able to reveal the earliest possible signs of toxicity, i.e., at a stage when the effects noted may not be completely irreversible. Unfortunately, we have not yet reached the level of sophistication in the technology for behavioral testing in animal subjects, where we can obtain valid and reliable answers quickly.

It is clear at the present time that there is a trade-off between speed and accuracy in the methods of behavioral toxicology. This is particularly apparent, for example, in the evaluation of hearing impairment following the administration of ototoxic agents to experimental animals. In the category of quick tests is the Preyer reflex, a brief movement of the pinna of the external ear in response to moderate or intense sound. It is an unconditioned reflex requiring no training of the ani-

* Department of Otorhinolaryngology, Psychology and Kresge Hearing Research Institute. University of Michigan, Ann Arbor, Michigan 48109.

mal subjects, no instrumentation, and no particular sophistication on the part of the experimenter. Unfortunately, the Preyer reflex is limited to certain species, particularly the rodents, and is not readily observable in the carnivores and primates. Further, the threshold of the reflex—the point at which it becomes clearly observable to the human eye—is well above behavioral threshold levels of acoustic stimulation. The reliance on the eye of an observer, particularly near the threshold of the reflex, where pinna movement may be marginal, casts serious doubt on the reliability of the reflex in the assessment of hearing. Further, the relationship of the Preyer reflex to behavioral threshold is not well established, and thus its validity as a measure of hearing is in question. Since the reflex is so much less sensitive than behavioral threshold it is of little or no value in the detection of slight hearing impairments that may forecast later, more serious hearing loss. For example, the effects of neomycin or dihydrostreptomycin, administered to the point of producing a significant hearing impairment, may continue long after treatment with the drug is discontinued. Early detection of hearing loss requires more sensitive procedures than the Preyer reflex test and yet, as with so many poisons, early detection may be critical in preventing later catastrophic effects. Other than speed and simplicity the Preyer reflex test has little to recommend it.

Other apparent indices of hearing suffer from some of the same problems. Classical conditioning procedures are not particularly quick to apply and the data are often not sufficiently stable to yield unequivocal measures of hearing or hearing loss. Physiological measures like the cochlear microphonic potential are usually carried out in an acute preparation, and, in any event, bear an uncertain relationship to actual hearing. In some instances, tests like the Preyer reflex may be applied where an estimate that hearing loss has occurred may be adequate, but it cannot be much more than an estimate, and the precise details with regard to the extent of the impairment require other, more sophisticated, and more time consuming methods.

Such methods are based on the technology of operant conditioning and require substantial investment in time and money. They are time-consuming, primarily because of the extensive training regimens necessary with animal subjects before stable behavioral baselines are obtained. Histological procedures for microscopic examination of the inner ear (*1*) yield accurate and quantitative analyses of the cellular changes produced by the various ototoxic agents. It is important that behavioral procedures be used which match the degree of resolution and rigor afforded by these anatomical procedures. An uncertain answer to the question of hearing loss is seldom adequate. It should be possible instead to offer a comprehensive evaluation of the extent of the hearing loss which can be readily and precisely correlated with the histopathological changes occurring in the cochlea of the inner ear.

In order to satisfy the demand for procedures that will provide answers quickly, it is tempting to cast around for a simpler index of hearing: one which is free of elaborate instrumentation and does not require intricate and extensive training of the experimental subjects. The search has not been successful. A viable alternative is to reduce the training time by improving the efficacy of the conditioning procedure we now use. As we will attempt to show, the behavioral baselines which these procedures provide meet the requirements of validity, reliability, and stability. The question then is whether we can increase speed without a loss in reliability.

In questioning animals about the limits of their sensory resolution we must first find some way of bridging the language gap between human experimenter and animal subject. Operant conditioning procedures supply this bridge. We expect much of them. To an extent, they must substitute for the lifetime of language training that man has had, and in a matter of weeks, transform a naive, even wild, animal into an attentive observer and reliable reporter of sensory events which are barely detectable. Can we further hasten the process? We will describe the procedures in current use, which include, in addition to the conditioning methods that have proved effective, the psychophysical methods used to determine sensory thresholds—minimum detectable levels of stimulation. Results will include a description of the normal baselines (auditory threshold function), in addition to the specific effects of the ototoxic antibiotics on the animal's ability to detect or resolve acoustic energy.

Training Procedures

Animal subjects in these experiments are trained to report a brief auditory signal so that their auditory acuity may be evaluated by varying signal intensity and noting the signal level just detectable, i.e., the threshold. Although the conditioning procedures for different animals are highly similar, the behavioral response is selected on the basis of the habits of the particular species chosen for study.

A fully conditioned monkey responds to a flashing light by placing one hand on a metal cylinder (Fig. 1). When he makes contact, the light stops flashing and becomes steady. The monkey continues to maintain contact until a tone is presented, whereupon he withdraws his hand, a food pellet is

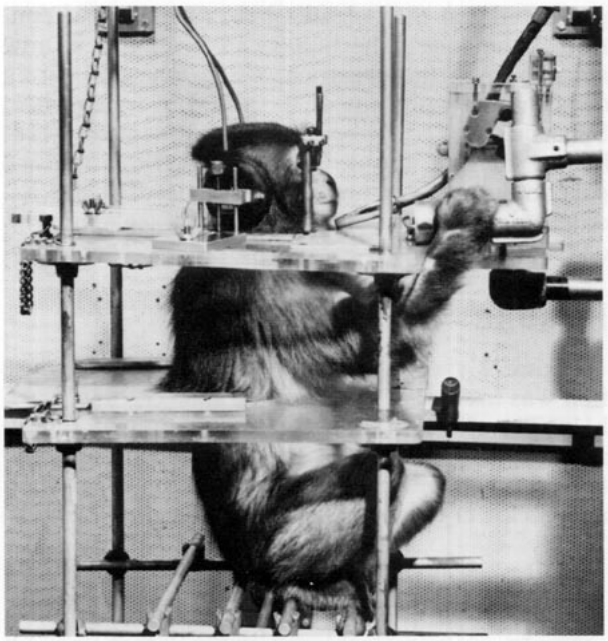

FIGURE 1. Subject, a macaque monkey, seated in restraining chair for hearing testing. Earphones, cylindrical response tube, and feeder tube are shown. The monkey is in listening position with his hand grasping the response tube prior to tone stimulation (*4*).

delivered to him, and both tone and light go off (*2*, *3*). Should he fail to hear the tone, he maintains contact with the tube until a signal that he can hear is finally presented. This straightforward behavioral sequence is the end result of a lengthy training procedure.

Following a quarantine period of 6 weeks, which includes a series of three TB tests and a number of routine health checks, the monkey is brought to our experimental colony and individually caged. He is fitted with a lightweight Plexiglas collar and chain and introduced to a primate restraint chair, as shown in Figure 1 (*3*). Initially, the animal is placed forcibly in the chair with the aid of a metal pole 1m in length that attaches to the collar. The use of the pole gives the handler greater leverage in controlling the movements of the animal and also protects him from being scratched or bitten. After the monkey adapts to the handling procedure and enters the primate chair readily, the metal pole becomes unnecessary, since the animal can be guided to the chair with the chain alone. Some animals are ready for direct handling in 2 weeks; others require a longer period. The Plexiglas collar still allows the handler a means of controlling the animal's movements. Eventually, through the use of food reinforcement, the monkey learns to enter the chair when his cage door is opened, and the Plexiglas collar is replaced by a leather collar.

During the period of chair training, the monkey is also acclimated to the laboratory, and introduced to some of the key elements included in the training regimen. While in the primate chair, he is hand-fed with the whole-diet banana-flavored pellets (Noyes, 190 mg, Formula L) that will later be used as food reinforcers in the conditioning procedure. When he readily accepts the pellets (usually within one or two days) a feeder chute is attached to the front of the chair and the animal quickly learns to pick up the individual pellets with his tongue as they are delivered down the chute. Next, a head restraint is introduced. The monkey's nose is positioned between two vertical metal bars connected by a metal nosepiece that prevents him from raising his head and turning out of position. A Plexiglas head restraint is then positioned behind his head. The head restraint ensures that the animal will be looking in one direction, and that in a later stage of training, earphones can be fitted securely over the openings to the external ear canals. He adapts to the head restraint fairly quickly and is next placed in the double-walled soundproof room where the hearing testing will be conducted. At this stage, the food pellets are dispensed by an automatic feeder. When he responds quickly to the sound of the feeder and consumes the food pellet, the formal conditioning procedure can begin.

In front of the monkey is a hollow metal tube containing a small red light (see Fig. 1). Initially, he is given food for simply reaching toward the response tube, and then for successive approximations to making manual contact with the tube. The animals are deprived of food for 24 hr before being introduced to this first step in behavioral conditioning. At this stage of the experiment some animals will require a longer period of food deprivation before they are ready for preliminary training. Particularly during this critical period, the animal's weight and general health are watched very carefully.

When the response of manual contact with the tube first occurs, a tone of mid-range frequency, clearly audible, is presented through earphones positioned close to the monkey's ears. If he releases the tube while the tone is on, he is reinforced with a food pellet. The sequence of events just described is referred to as a trial and begins with the flashing red light in the response tube. This signals the animal to respond. When he effectively makes manual contact with the tube the light stops flashing; when he breaks contact the light goes out. If the monkey fails to let go of the tube while the tone is on, another tone is presented after a variable time period. The standard tone duration is 2.5 sec, although in the

first training session it may be as long as 15 sec. The time required for holding the response tube before the tone is presented is very short early in training (perhaps less than 0.1 sec). When the monkey consistently makes contact with the key upon being presented with the flashing light and breaks contact in response to the tone, the time that he is required to maintain contact with the tube before the tone is presented is gradually lengthened so that it varies between 1 and 9 sec. If he releases before a tone is presented or if he makes contact with the key before the flashing light comes on, a time-out ensues and the onset of the next trial is delayed. The time-out period is relatively short when shaping begins (1 sec), but is later increased to 5 sec. The lengths of these various time intervals are increased as the training progresses, and may have to be decreased if the animal's behavior is disrupted or regresses for some reason (5). Occasionally a very long time-out period will be employed as a form of punishment for inappropriate key releases. The standard intertrial interval is 5 sec. The final sequence, then, is: flashing light, contact, steady light, hold, tone presentation, release, reinforcement.

The earphones are now positioned directly over the monkey's ears and fastened to the neckplate of the Plexiglas chair (see Fig. 1). Coupling the earphones tightly against the ears permits us to specify the characteristics of the signal (particularly its amplitude) as accurately as possible. By using earphones (as opposed to free-field presentation) we can test each ear separately, an important advantage in studying the effects of ototoxic agents. To ensure that he is responding to the tone and not randomly releasing the response key, a number of catch trials (i.e., trials in which no tone is presented) are introduced. If the monkey responds positively to a catch trial, and thereby inappropriately releases the response key, a time-out period ensues. Until the animal responds to less than 10% of these catch trials, the data are considered unreliable and further training is necessary. When responding occurs to 95% or more of the tones presented, the monkey is considered ready for threshold testing. It may take anywhere from 4 to 12 weeks for him to reach this stage of training. This procedure has proven effective; we have never had to discard an animal because it was untrainable. In the course of training and later hearing testing, the animals earn most of their daily food allowance during the experimental sessions. They are weighed regularly and given repeated health checks. Food and vitamin supplements are given after the sessions so that the animals enter each daily session hungry.

The conditioning methods are designed to guarantee that the animal's behavior is under control of the tone. The behavioral response is now made only in the presence of the tone and rarely (less than ten precent of the time) in its absence. Subsequently, to probe the limits of acoustic resolution of the monkey, one of the standard psychophysical methods that are used in the audiometric examination of human subjects is typically employed.

Results

Figure 2 shows results of a tracking procedure that was used to determine pure tone threshold at 8 kHz. The stimulus is progressively attenuated from a clearly audible level as the monkey correctly detects it on each successive presentation. Finally, at a much lower level of stimulation, the animal fails to

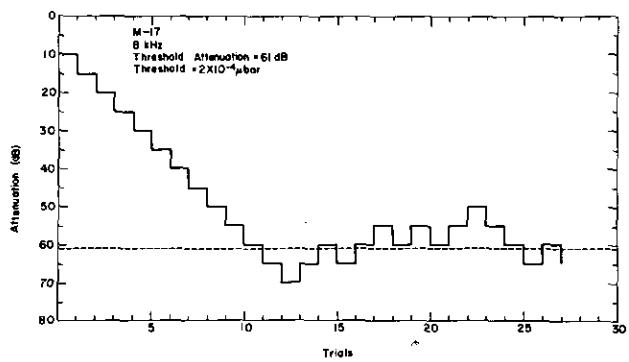

FIGURE 2. Use of the tracking method for audiometric testing of monkeys. Correct detections cause the tone to be attenuated in 5 dB steps, while failure to hear produces a subsequent 5 dB increase in tone intensity (2).

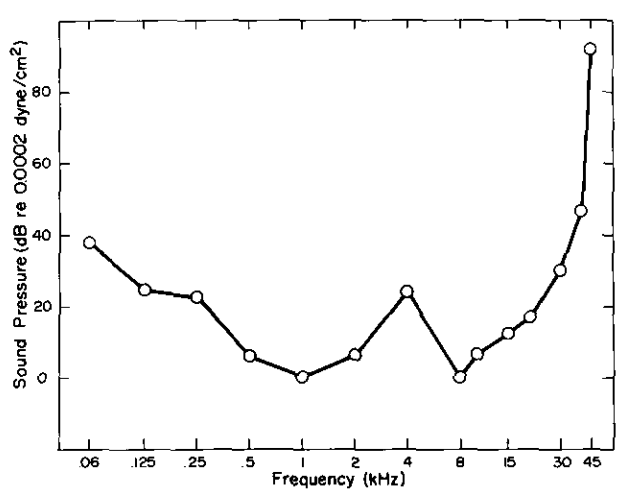

FIGURE 3. Threshold of hearing function for one macaque monkey (4).

respond and the direction of stimulus change is reversed—the intensity of the tone is increased the next time it is presented. Since the stimulus is now close to threshold, reversals of stimulus intensity occur frequently and the threshold itself, which is a statistical measure, is based on eight such reversals; it is, in fact, the stimulus level to which the subject responds correctly on 50% of the trials. Threshold determination at a single pure tone frequency takes about three minutes.

A complete audiogram—that is, thresholds determined at seven to ten frequencies in each ear is accomplished in close to 1 hr in a well-trained animal. A typical threshold curve for an Old World monkey can be seen in Figure 3, in which sound pressure level at threshold is plotted as a function of pure tone frequency. There is a strong similarity to the audibility function for man, which is clearly important if we are to consider the Old World monkey a worthy model for the evaluation of ototoxic agents administered to man. The only significant departure in the audibility functions is the extension of the monkey's hearing in the high frequency range to about 40 or 45 kHz, i.e., about an octave above the upper limit for man. Further, there is a definite correspondence in the morphology of the peripheral auditory system in man and monkey.

Figure 3 then represents the normal pretreatment baseline for the monkey. It is against this baseline that we measure the effects of agents which may be ototoxic. By audiometric convention, normal threshold stimulus values at all frequencies are set to zero decibels (0 dB), and hearing loss (threshold shift) is measured as an increase in sound intensity required for hearing—plotted in a downward direction. In Figure 4, for example, hearing loss is shown advancing over a time span of 6–7 months at several test frequencies. The ototoxic antibiotic kanamycin was administered to a macaque monkey and thresholds were examined daily. In our experience, increased variance in the data, as indicated by marked fluctuations in the thresholds during the first month of treatment, is often a precursor of moderate to severe hearing loss. Hearing loss following treatment with the ototoxic antibiotics is permanent and the progressive nature of this impairment can be seen clearly in Figure 4. The highest frequencies are the first to go as in presbyacusis (high frequency hearing loss due to aging), but the time frame is much briefer. Eventually the lower frequencies including those in the human speech range, for example, become involved, and if the treatment is continued complete deafness may ensue. For the monkey whose hearing is described in Figure 4 antibiotic treatment was stopped after 180 days. Audiometric testing continued for some time after the termination of drug treatment in order to observe any possible delayed loss which might occur. The terminal audiogram for each ear (before the animal was sacrificed for histology) is shown in the lower panel of Figure 5. This animal was completely deaf to frequencies of acoustic

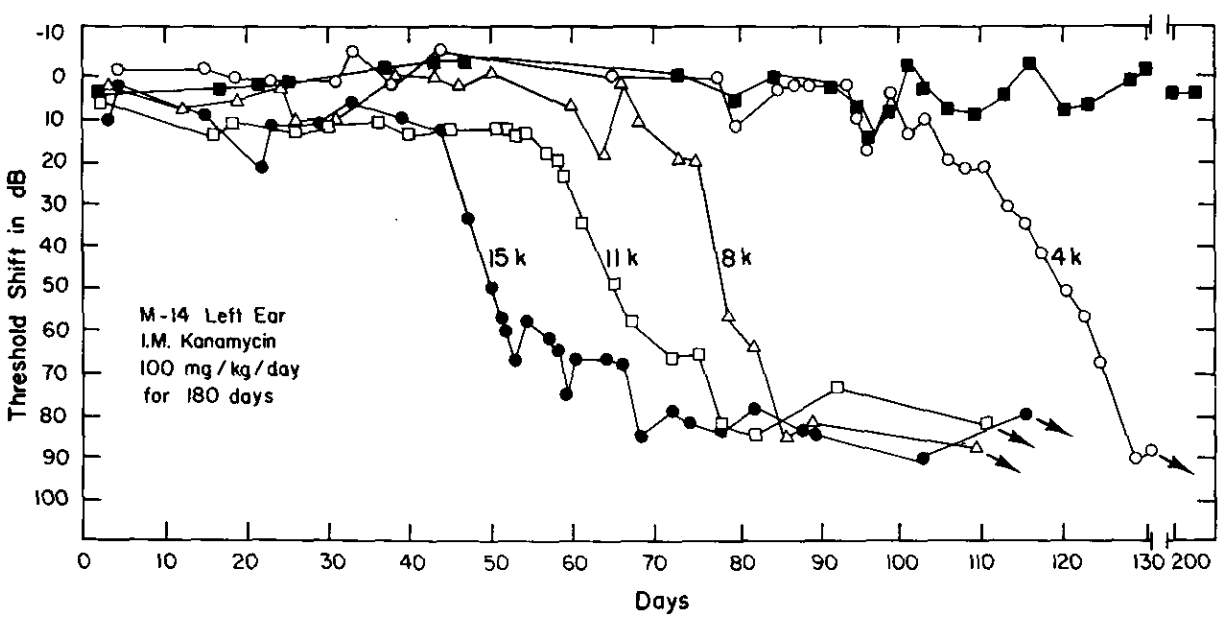

FIGURE 4. Progressive changes in threshold for one macaque monkey for different acoustic frequencies during daily kanamycin treatment. The zero line represents normal hearing at all frequencies prior to drug treatment (6).

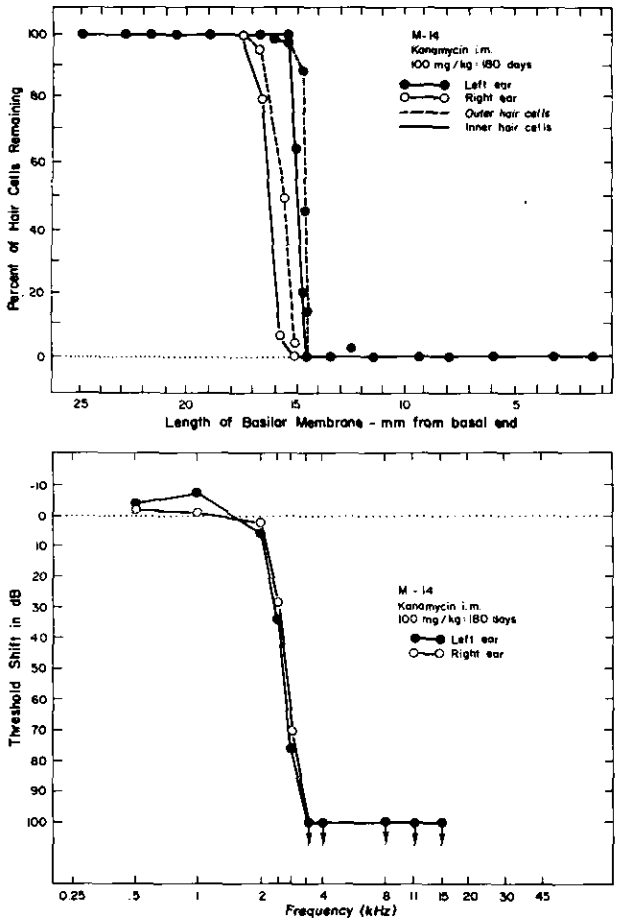

FIGURE 5. Auditory threshold shift for the monkey represented in Fig. 4 after 180 days of daily kanamycin (lower panel); hair cells remaining as a function of position on basilar membrane (upper panel) (*2*).

stimulation above 3.4 kHz, yet its hearing was normal to frequencies below 2 kHz. The hearing loss was identical in the two ears—a common observation in aminoglycoside ototoxicity. The sharp transition between normal hearing and complete impairment is also characteristic of such ototoxicity.

When the animals are sacrificed their temporal bones are taken for microdissection of the cochlea of the inner ear in preparation for its examination and evalution by phase contrast microscopy (*1*). Inner and outer hair cells that are still present are counted, and cytocochleograms are constructed as shown in the upper panel in Figure 5. The juxtaposition of the scales on the abscissa for the behavioral and morphological data, although arbitrary, is standardized for each species that we have studied. The correspondence between the pattern of hair cell loss on the basilar membrane of the cochlea and the frequency specificity of the hearing loss is evident in this figure. The sharp boundary on the basilar membrane between normal appearing receptor cells and the absence of these cells is correlated with the equally sharp transition between 2 kHz and 3.4 kHz in the audiometric data (lower panel, Fig. 5). The two sets of data, behavioral and morphological, complement each other in providing compelling evidence regarding the nature of the ototoxic process. As will be discussed later, these data lend strong support to the theoretical notion regarding the coding of acoustic frequency in the inner ear by place of stimulation along the basilar membrane containing the receptor cells.

The audibility or absolute threshold function is but one way of behaviorally assessing the status of the auditory system. The information it provides is a measure of the minimum detectable energy levels that the system is capable of resolving. In addition, it is important to consider the resolution of the system at higher levels of stimulation that are clearly audible to the normal ear. One such measure is the frequency difference threshold Δf, which is the least difference between two acoustic frequencies to which an animal can respond. The testing procedure is very similar to the one described for the determination of minimum detectable levels of stimulation. The animal is first trained to respond to widely differing frequencies. By use of the tracking method two frequencies are brought closer and closer together until he is unable to distinguish between them. Normal frequency difference thresholds determined in this manner are shown in Figure 6. These data are similar to those obtained for man

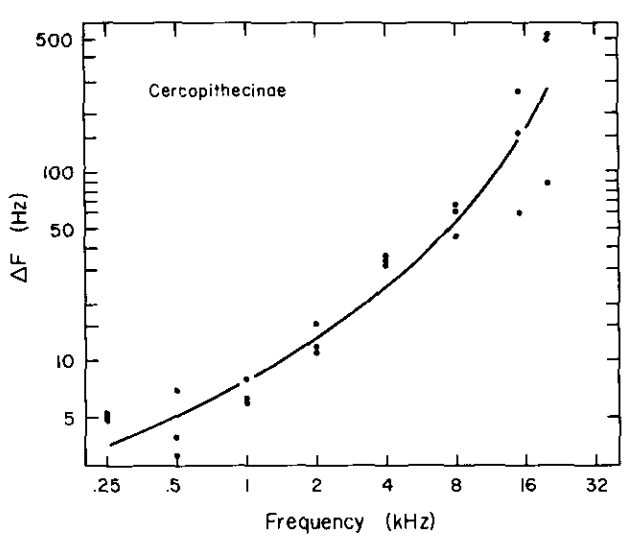

FIGURE 6. Frequency difference thresholds at 60 dB above threshold as a function of frequency for three subjects (macaques) (*7*).

indicating a decrease in resolution for frequency at higher frequencies of acoustic stimulation.

It is feasible to train an animal on two different baselines, and, in the context of evaluation of ototoxicity, two different measures of hearing may then be examined in the same subject. An example is shown in Figure 7 for a monkey treated with kanamycin and tested on alternate days for absolute intensive threshold and differential frequency threshold. The typical high-frequency hearing loss is apparent, as is the sharp transition between normal hearing and complete impairment. Of interest in this example is the observation that the animal's ability to resolve small differences in acoustic frequency is affected, and this progressive deterioration in frequency resolution precedes the predicted changes in absolute intensive threshold. Such findings indicate the importance of looking at more than one aspect of the hearing process, since toxic effects may be differential. It is standard procedure in the clinical audiological examination to include several measures of hearing. We have adapted several of these tests for use in the animal laboratory (*5*).

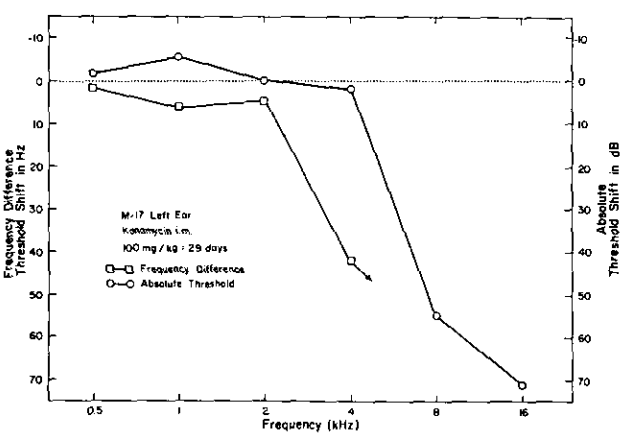

FIGURE 7. Changes in absolute auditory threshold and frequency difference threshold in macaque monkey treated with kanamycin (*8*).

As an animal model the Old World monkey has certain obvious advantages over the nonprimates, such as its close phylogenetic relationship to man and the strong similarity to man in hearing and inner ear structure. The disadvantages of using monkeys in behavioral ototoxicology studies, although not of a scientific nature, are equally obvious. These animals are expensive to purchase, and many are beginning to appear on the lists of endangered or threatened species. It is not always amply clear why nonhuman primates are selected for particular experiments. If we can adequately defend their status as animal models, other animals may serve as well. In experiments on hearing, guinea pigs (*9, 10*), cats (*11*) and chinchillas (*12*) have proven viable subjects. They can be questioned about their sensory experiences no less rigorously than monkeys and their answers are equally reliable and valid. We have used somewhat similar operant conditioning and psychophysical procedures in obtaining audibility functions from these animals. In addition, we have evidence that they react in a similar manner to treatment with certain ototoxic substances. Progressive hearing loss from the high to the low frequencies with continued kanamycin treatment in

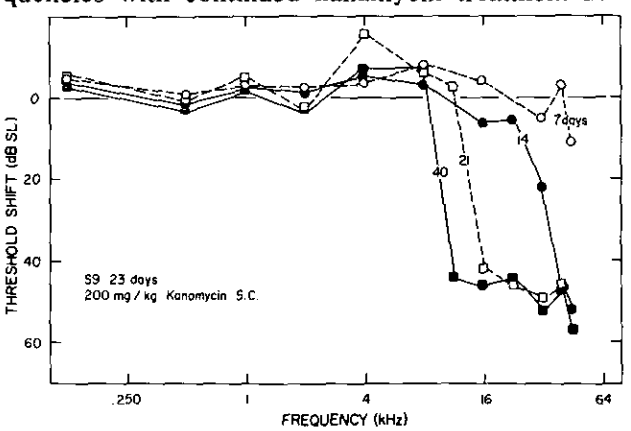

FIGURE 8. Auditory threshold shift measured 7, 14, 21, and 40 days after the initial treatment with dihydrostreptomycin in one guinea pig (*10*).

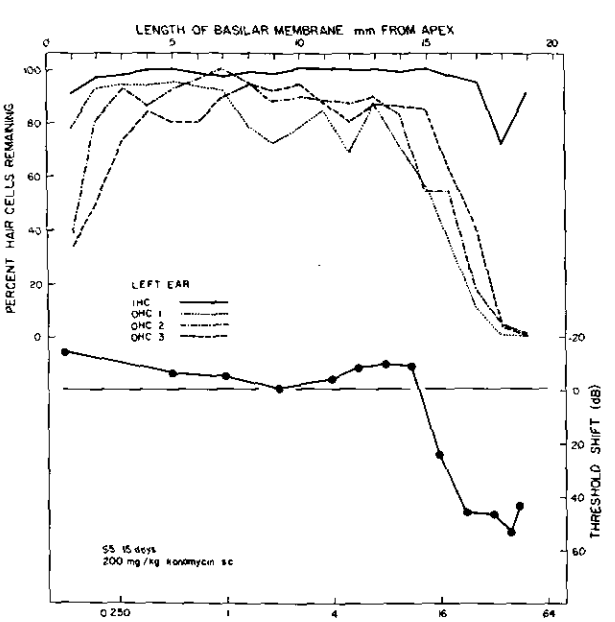

FIGURE 9. Cytocochleogram and auditory threshold shift, measured five weeks after last treatment with dihydrostreptomycin for one guinea pig. The pattern of hair cell loss was symmetrical in both ears (*10*).

one guinea pig is shown in Figure 8. The relation of hearing loss to the histopathological changes which occur in the inner ear are shown for another animal in Figure 9. As in the monkey, there is a sharp gradient between normal and impaired hearing at the higher frequencies reflected in a loss of outer hair cells in the basal turn of the cochlea. In contrast to the monkey, the inner hair cells in the guinea pig's ear are relatively less affected.

Serendipitously, we have discovered an unusual instance of what appears to be species-specific toxicity. The patas monkey (*Erythrocebus patas*), like the macaque, is an Old World monkey, although African rather than Asian. However, unlike the macaque, the patas is extremely sensitive to the aminoglycoside antibiotic dihydrostreptomycin (*13*). Such findings point to the highly specific yet poorly understood effects of some poisons and underscore again both the importance and the difficulty in selecting appropriate animal models in behavioral toxicology. In our experiments, six macaques were treated daily for as long as 8 months with dihydrostreptomycin at dose levels as high as 100 mg/kg (five times the clinical dose). Only one animal showed a marginal hearing loss, i.e., less than 40 dB at the highest frequency tested (32 kHz). Seven patas monkeys were treated at dose levels ranging from 20 mg/kg to 100 mg/kg; all showed severe hearing loss accompanied by extensive histopathological changes in the inner ear. Certain features of this loss are evident in Figure 10, which illustrates the audibility function in each ear for a patas monkey at one week and at 13 weeks after drug treatment had ceased. The initial high-frequency loss and the fairly sharp gradient between normal and impaired hearing, typical of aminoglycoside ototoxicity, can be seen in the one week functions to the right of the figure. As dramatic and also typical of this form of hearing loss is the remarkable symmetry in the response of the two ears, both at 1 week and at 13 weeks. The symmetry is also reflected in the pattern of hair cell loss along the basilar membrane (*13*). More peculiar to dihydrostreptomycin is the delayed, progressive hearing loss continuing long after the antibiotic treatment has stopped. Although hearing had been affected primarily at the high frequencies and was essentially normal over a wide range of frequencies at the end of treatment, almost no hearing remained 3 months later. Such delayed losses have been reported in some tuberculous patients treated with dihydrostreptomycin.

The relation between hearing loss and hair cell loss is shown in another patas in Figure 11. Again a delayed effect of the drug after the end of treatment is seen. The sharp boundary between normal and

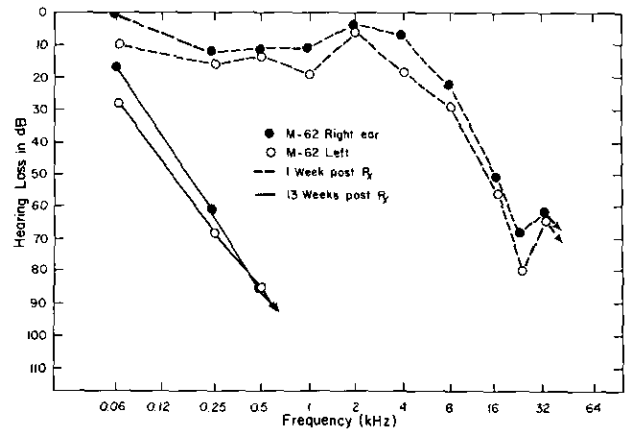

FIGURE 10. Auditory threshold shift for one patas monkey after daily treatment with dihydrostreptomycin. The functions to the upper right represent the hearing loss in each year one week following cessation of drug treatment. The functions to the lower left represent the hearing thresholds 12 weeks later (*13*).

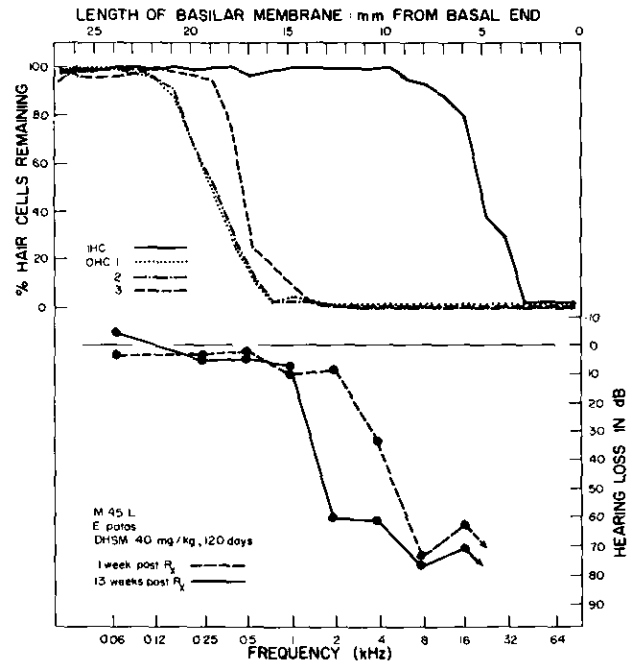

FIGURE 11. Auditory threshold shift and cytocochleogram for one patas monkey treated with dihydrostreptomycin. The dashed function represents hearing loss one week after drug treatment was stopped; the solid function indicates hearing loss twelve weeks later (*13*).

impaired hearing is reflected in the pattern of outer hair cell loss in the organ of Corti of the inner ear. Although few outer hair cells remain, the inner hair cells are still present except near the basal end of the basilar membrane. This pattern in the ear of the dihydrostreptomycin-treated patas monkey resembles that in the kanamycin-treated guinea pig. The

kanamycin-treated macaque monkey, on the other hand, shows no such differential loss between outer and inner hair cells, both disappearing at about the same rate.

These data strengthen and confirm earlier theoretical formulations concerning stimulus coding in the mammalian ear. Clearly, frequency coding according to a place mechanism is indicated by the relationship of the hearing threshold function and the hair cell loss function in all of the drug treated animals. In some way not yet completely understood, high frequencies are represented in the more basal regions of the cochlea and lower frequencies more toward the apex. Further, it has been suggested that the outer cells are somehow responsible for normal hearing near threshold, while inner hair cells respond only at high intensities of stimulation (*10, 14*). The value of such toxicological studies for revealing the mechanisms which underly normal function obviously provide an additional justification and interest in doing such experiments.

Summary

In this paper we have tried to illustrate, by example, the efficacy of a set of procedures for evaluating the behavioral effects of substances which are toxic to the ear. Although the methods are time-consuming (training may take one month or more), it is unlikely that there are alternative, more rapid procedures that are as valid and reliable and afford the degree of resolution necessary for accurate assessment of ototoxic agents. As we have noted, "quick and dirty" methods are available and although they may, on occasion, provide quickly-arrived-at estimates of potential toxicity, they may equally often yield equivocal findings or even misinformation. Further, due to greater variance such estimates often require prohibitively large numbers of subjects.

The procedures we have described are based largely on operant conditioning and classical psychophysics. Our examples are taken from hearing, but the procedures themselves are applicable to the behavioral study of sensory systems generally or the analyses of toxicants which influence any aspect of sensory or perceptual processing. They are moderately complicated and require both professionally trained personnel and sophisticated instrumentation but they are applicable to a wide variety of animal subjects. They have the further advantage of permitting us to question an animal in considerable detail about a wider range of sensory and perceptual capabilities. Information not only concerning the limits of response of the sensory system (thresholds) but also such characteristics as frequency resolution and the discrimination of stimuli well above the threshold region may be obtained with the procedures of animal psychophysics (*15*).

Finally, we have tried to show that these behavioral methods when used with methods that reveal the morphological changes occurring in response to toxic agents, can lead to a better understanding of the basic mechanisms underlying abnormal and normal auditory function.

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