Brain Research, 187 (1980) 81–95
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ACOUSTIC PRIMING AND KANAMYCIN-INDUCED COCHLEAR DAMAGE

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(Accepted August 23rd, 1979)

Key words: acoustic priming — auditory system — cochlear hair cell pathology — kanamycin — supersensitivity

SUMMARY

The cochleae from 3 lines of mice, selectively bred for differential susceptibility to priming-induced audiogenic seizures, were examined following acoustic priming and retest or kanamycin treatment, and the degree of cochlear damage was assessed. After 60 sec of acoustic priming, animals from the high and unselected lines which had subsequently developed audiogenic seizure susceptibility exhibited severe cochlear damage limited to the outer hair cells. Low line mice, which had been selected for resistance to acoustic priming-induced audiogenic seizures and were not seizure susceptible, exhibited no cochlear pathology following acoustic priming.

Following kanamycin treatment, all 3 lines developed subsequent audiogenic seizure susceptibility. Histological examination of cochleae from mice so treated revealed a pattern of damage similar to that caused by acoustic priming, except that the cochleae of priming-induced audiogenic seizure resistant low line mice revealed a significant amount of outer hair cell damage.

The results are discussed with respect to the physiological mechanism underlying a selectively bred behavioral phenotype in terms of a possible instance of damage/disuse-supersensitivity in the central nervous system.

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Mice genetically resistant to audiogenic seizures can be rendered susceptible to sound-induced convulsions by exposure to an intense auditory stimulus during a critical period of neural maturation. This phenomenon has been called *acoustic*

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INTRODUCTION

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priming, and was first described by Henry[11]. The priming effect is constrained by both temporal and genetic factors. There appears to be a critical period during which the application of the priming stimulus is efficacious in rendering animals susceptible to audiogenic seizures[1,12], much as there appears to be a critical period for the elaboration of first-trial audiogenic seizures in mice of certain genotypes[20]. Genetic factors appear to be important in acoustic priming since it is easier to prime animals of certain genotypes than others, and since it is possible to selectively breed animals for acoustic priming efficacy[7,9].

Since acoustic priming was first described, a considerable research literature has accumulated in an attempt to describe some of the causal mechanisms underlying this phenomenon. There now exists both behavioral and physiological evidence which tends to support the hypothesis that acoustic priming is an instance of damage-induced supersensitivity. This explanation was first proposed by Saunders, Bock, James and Chen[18], and by Sharpless[22,23]. According to this hypothesis, the intense auditory stimulus administered when the animal is first primed damages some cochlear elements. This results in reduced afferent input to higher neural structures producing disuse supersensitivity, such that when animals which have been primed are re-exposed to an intense acoustic stimulus, a massive afferent volley originating in the still intact cochlear elements triggers a convulsive response. Evidence which supports this hypothesis comes from the following types of experiments: damage to the tympanic membrane, which results in auditory deprivation, mimics acoustic priming in that it increases susceptibility to audiogenic seizures[4,10]; reducing auditory input by plugging the ear with cotton and clay increases the incidence of audiogenic seizures[15]; acoustic priming diminishes the amplitude of the cochlear microphonic and auditory nerve responses to subsequent auditory stimulation[17]; and the auditory projections from the cochlear nucleus to the inferior colliculus show diminished responses to weak sounds and increased responses to intense sounds, suggesting that the auditory system experiences a paucity of input from low-level noise but reacts rather explosively to high-level noises[13,17,28].

Aminoglycoside antibiotics are ototoxic when administered chronically in high doses. In particular, kanamycin has been shown to be extremely ototoxic, causing the destruction principally of outer hair cells in the basal region of the cochlea. Increasing the dosage of the drug and the length of treatment results in the extension of the damage to the apex of the cochlea and inner hair cells may also be destroyed[3]. Based on the damage-induced supersensitivity explanation proposed to account for acoustic priming, one might therefore predict that kanamycin treatment should mimic the effects of acoustic priming. Indeed, Norris, Cawthon and Carroll have shown that kanamycin treatment renders animals extremely susceptible to audiogenic seizures[16].

The purpose of the experiments reported here was to examine the effects of acoustic priming and of kanamycin treatment on auditory receptor cells. With respect to the effects of these treatments we made the following specific predictions: (1) auditory stimulation will render animals from the line selectively bred to exhibit acoustic 'primability' susceptible to subsequent audiogenic seizures; in these animals, auditory stimulation will produce cochlear hair cell damage. Auditory stimulation will

not render animals from the line selectively bred to be resistant to this treatment acoustically primed; in these animals, auditory stimulation will not produce cochlear hair cell damage; and (2) kanamycin treatment will render animals from all lines audiogenic-seizure susceptible; treatment with this antibiotic will produce cochlear hair cell damage in all animals.

METHODS

Subjects

High, low and unselected mice from the 17th, 18th and 19th generations of the selective breeding experiment for priming-induced audiogenic-seizure susceptibility described by Deckard et al.[7] were used as subjects. Briefly, these mice are derived from a base population of heterogeneous stock (HS) mice bred from an 8-way cross of inbred strains. The origin of these HS mice is described more fully by McClearn, Wilson and Meredith[14]. The average seizure severity scores for all 3 lines are not significantly different from those reported for the 8th selected generation by Deckard et al.[7]. During the course of these experiments animals were maintained under standard laboratory conditions of temperature (74 $\pm$ 3 °F), and controlled lighting (12 h light cycle, 06.30–18.30 h) with ad libitum access to Purina Mouse Breeder Chow and tap water.

Acoustic priming and retesting

Pups were weaned at 19 days of age and litters were divided randomly into two groups. One half of the litter was individually placed into a large glass chromatography jar measuring 45.5 cm in height and 29.5 cm in diameter. After a 10-sec adaptation period, an electric bell mounted over the chromatography jar was sounded for 60 sec. This 5-inch electric bell delivered approximately 116 $\pm$ 3 dB of broadband noise at the level of the mouse. A spectral analysis of the bell was performed utilizing a Bruel and Kjaer calibrated condenser microphone (model no. 4136) in conjunction with a Tektronix spectrum analyzer. The sound spectrogram appears in Fig. 1. During

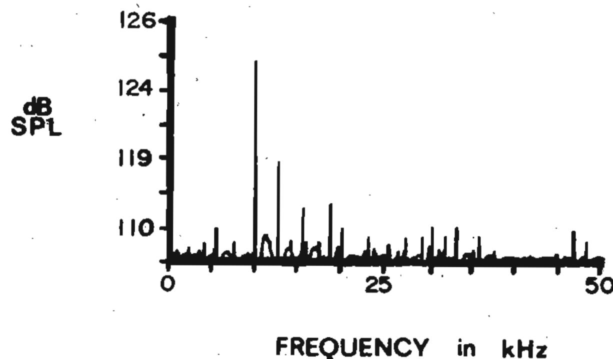


Fig. 1. This shows a spectral analysis of the priming/retest apparatus. Recordings were made with a Bruel and Kjaer calibrated condenser microphone (model no. 4136) situated 4 cm from the sides and 2 cm from the base of the chromatography jar. The preamplified output of the microphone was input to a Tektronix spectrum analyzer and the power output of the stimulus was recorded as a function of frequency from 0–50 kHz.

this period, the animals were observed and records made of the incidence of wild running (WR), clonic seizures (C), tonic seizure (T), and lethal seizures (L). After priming, animals were placed into clean cages separated on the basis of the litter from which they came, their sex, and treatment (primed or non-primed). The remaining one-half of each litter was sham-primed; the animals were placed into the chromatography jar for 60 sec without the bell being sounded. All priming and retesting procedures were done between the hours of 09.00 and 15.00 h.

At 22 days of age, all animals were given an audiogenic seizure test identical to the previously described acoustic priming procedure. Records were taken as described above. For purposes of statistical analyses, each mouse was assigned a seizure severity score based on its response: no response (NR) = 0; wild running (WR) = 1; clonic seizure (C) = 2; tonic seizure (T) = 3; and lethal seizure (L) = 4. In the event a lethal seizure developed upon retest, the animal was revived through artificial resuscitation. We were successful in reviving more than 90% of animals exhibiting a lethal seizure. After the retest animals were placed into separate cages based on their litter, sex, treatment (primed or non-primed) and retest seizure score.

Kanamycin priming and retest

Beginning at 10 days of age, each litter was randomly divided into two groups, kanamycin or control. Animals in the kanamycin group were weighed, and received 312.5 mg/kg kanamycin sulfate in normal saline, i.p., in a vol. of 0.01 ml/g body weight. Animals in the control group received saline, i.p., in an equivalent vol. After injection all pups were returned to their home cages. Weighing and injections continued once daily through weaning at 19 days of age, and were discontinued after the injection on the 21st day. All animals were given an audiogenic seizure test on the 26th day precisely as described in the preceding section, and the incidences of WR, C, T and L seizures, and a seizure severity score for each mouse were recorded.

Histological preparations

At 30 days of age, all mice were sacrificed by decapitation and the cochleae removed essentially as described by Engstrom, Ades and Andersson[8]. The scalp was reflected to reveal the temporal bones on each side and soft tissues cleaned away. The bulla was removed and the temporal bones cut out of the skull. Under a stereomicroscope the stapes was removed, and the round and oval windows opened with a fine pair of forceps. Bone was chipped away from the apical crest of the cochlea, and the cochlea (with the temporal bone still attached) was dropped into a solution of 3.7% glutaraldehyde in 0.12 M (260 mOsm) phosphate buffer. The entire procedure up to this point took from 2 to 3 min and was always timed.

The cochleae were perfused in 3.7% glutaraldehyde for 24 h at 2 °C. They were then washed 3 times with 0.12 M phosphate buffer and immersed in 1% osmium tetroxide in 0.12 M phosphate buffer for 24 h at 2 °C. Cochleae were then rinsed 3 times with polished water and stored in polished water at 2 °C until dissection.

Cochleae were dissected under water, viewed through a stereomicroscope at 30 × magnification. The temporal bone was used as a handle to grasp the cochlea and the

bony labyrinth was carefully chipped away with a 26-gauge hypodermic needle and Dumont no. 5 forceps. The stria vascularis was removed and the tectorial membrane was very carefully pulled off. The cochlea was divided into 3 sections as follows: (1) the basal section consisted of that portion of the basilar membrane extending from the oval window ascending to midway around the first turn; (2) the middle section consisted of the basilar membrane extending from this point to midway around the second turn, and (3) the apical section consisted of the basilar membrane extending from this point to the apical end of the cochlea. Each cochlea was so divided with fine forceps, and each cochlear section placed in a drop of polished water on a microscope slide previously dipped in a 1 % gelatin solution and placed on a slide warmer at 40 °C to dry. Slides were then dehydrated, cleared in 100% xylene for 15 min, and mounted in synthetic resin. Slides were viewed by phase contrast light microscopy, and the degree of pathology assessed. Cells were considered damaged if they were missing, markedly distorted in size or shape, enucleated or showed signs of cytoplasmic shrinkage or total cellular collapse.

RESULTS

Behavioral effects of acoustic priming

The average seizure severity scores for primed and control animals from the three lines are shown in Table I.

Acoustic priming was found to be highly effective at inducing audiogenic seizure susceptibility in mice from the high ($F = 108.05$, df $= 1/94$, $P < 0.001$) and unselected ($F = 26.46$, df $= 1/112$, $P < 0.001$) lines, but not in the animals from the low line when compared to their respective non-primed controls. High-line mice were found to exhibit significantly more frequent and more severe seizures than unselected line mice ($P < 0.05$), and unselected-line mice were found to exhibit significantly more frequent and more severe seizures than low line mice upon retest ($P < 0.05$).

Behavioral effects of kanamycin treatment

The average seizure severity score for kanamycin-treated and control mice from the 3 lines is given in Table II.

TABLE I

Effects of acoustic priming on susceptibility to audiogenic seizures in mice selectively bred for differential acoustic priming efficacy

Numbers reported are average seizure severity scores ± 1 S.E.

Selected line	*Condition*			
	Not primed		*Primed*	
	N	$\bar{X} \pm 1$ *S.E.*	*N*	$\bar{X} \pm 1$ *S.E.*
High	36	1.03 ± 0.26	60	3.67 ± 0.12
Unselected	22	0.05 ± 0.05	92	1.57 ± 0.05
Low	19	0.00 ± 0.00	223	0.38 ± 0.06

TABLE II

Effects of kanamycin on susceptibility to audiogenic seizures in mice selectively bred for differential acoustic priming efficacy

Numbers reported are average seizure severity scores ± 1 S.E.

Selected line	*Condition*			
	Control		*312.5 mg/kg kanamycin*	
	N	$\bar{X} \pm 1$ *S.E.*	*N*	$\bar{X} \pm 1$ *S.E.*
High	25	0.52 ± 0.23	34	2.76 ± 0.25
Unselected	14	0.21 ± 0.15	21	1.38 ± 0.24
Low	32	0.00 ± 0.00	28	2.04 ± 0.17

Chronic kanamycin treatment was highly effective at inducing audiogenic seizure susceptibility in animals at all 3 lines. Statistical comparisons between kanamycin-treated and control animals indicate that this effect was statistically significant; the respective F ratios for animals from the high, unselected and low lines were $F = 40.4$, df = 1/57, $P < 0.001$; $F = 12.9$, df = 1/33, $P < 0.001$; and $F = 171.2$, df = 1/59, $P < 0.001$.

Effects of acoustic priming and retest on the cochlea

Sixty sec of acoustic priming at 19 days of age followed by a 60-sec retest at 22 days of age was sufficient to cause severe degenerative changes in the basal and middle 2/3 of the cochleae in mice of the high and unselected lines that were sacrificed at 30 days of age. The same procedure produced no visible damage anywhere in the cochleae of low-line mice. The control procedure wherein naive animals were given one 60-sec seizure test at 22 days produced no damage in cochleae of any of the 3 selected lines. In acoustically primed and retested animals of the high and unselected lines, the pattern of damage was similar to that reported in BALB/c mice after acoustic priming[16] (see Figs. 2–5). Damage was principally to the outer hair cells, being most severe and widespread in the outermost row, and less severe in the middle and innermost rows. Typically, all 3 rows of outer hair cells were affected to some degree in the basal portion of the cochleae examined. Pillar cells were only occasionally seen to be damaged, damage occurring most frequently in specimens where a tear in the organ of corti was observed. Inner hair cell damage was exceedingly rare and was observed only in two specimens, one from an acoustically primed mouse and one from a kanamycin-treated animal. When it did occur, it consisted of a single damaged or missing cell amidst other intact inner hair cells, in sharp contrast to the outer hair cells where massive damage was observed across large regions of the organ or corti. Two general classes of abnormalities among outer hair cells were observed within the cochleae of primed and retested seizure-susceptible high- and unselected-line mice: (1) missing outer hair cells; and (2) damaged outer hair cells in various stages of degeneration. Missing outer hair cells were observed far less frequently than damaged cells and were frequently surrounded by healthy intact outer hair cells. The site formerly occupied by

a missing cell was often replaced by a phalangeal scar resulting from the invasion of the vacant site by the phalangeal processes of the supporting Dieters' cells. More commonly observed were cells in the process of degeneration. Due probably to cytoplasmic shrinkage and the partial adherence of the outer hair cell membrane to supporting structures, the cells appeared to have collapsed inwards. In the extreme, most or all of the cytoplasm and nuclear structures had disappeared leaving a web-like network of membranes and cellular debris. Nuclei of damaged outer hair cells were frequently absent or very swollen.

The effects of acoustic priming and retest on outer hair cells are summarized in Table III. In mice of the high and unselected lines, the damage was most severe in the basal 1/3 of the cochlea where approximately 2/3 of the outer hair cell population was missing or damaged. The severity and incidence of the damage was less in the middle portion of the cochlea and the apical 1/3 of the cochlea was free of damage.

Representative photomicrographs obtained from the basal 1/3 of the cochleae of primed and retested high- and low-line mice and their respective controls are shown in Figs. 2–5. All micrographs are focused at the level of the reticular lamina. Fig. 2 is taken from a control unprimed but retested high-line animal (score = 0). Three rows of outer hair cells are present along with the heads of the outer pillar cells. There is no damage induced by the retest procedure alone at 22 days of age. Note the presence of the nuclei of row 3 outer hair cells near the top of the micrograph. Fig. 3 is taken from a high line animal primed at 19 and retested at 22 days of age (score = 4). Focus is at the level of the reticular lamina although some outer hair cell nuclei are also in focus. There is massive destruction of most outer hair cells judging from the appearance of the apical ends of the cells. Two outer hair cells exhibited almost normal appearance at

TABLE III

Effects of acoustic priming on cochlear hair cells

N refers to the number of cochlea examined. Per cent damage refers to the ratio of missing or damaged hair cells to total hair cells observed. On the average between 50 and 350 outer hair cells were observed per section.

Selected line	*Portion of cochlea*	*Per cent outer hair cell damage*			
		*Not primed**		*Primed*	
		N	*% Damage*	*N*	*% Damage*
High	Basal portion	4	0	9	78
High	Middle portion	3	0	10	47
High	Apical portion	5	0	6	0
Unselected	Basal portion	4	0	4	46
Unselected	Middle portion	4	0	3	21
Unselected	Apical portion	3	0	4	0
Low	Basal portion	4	0	4	0
Low	Middle portion	6	0	6	0
Low	Apical portion	5	0	5	0

* Seizure severity score = 0.

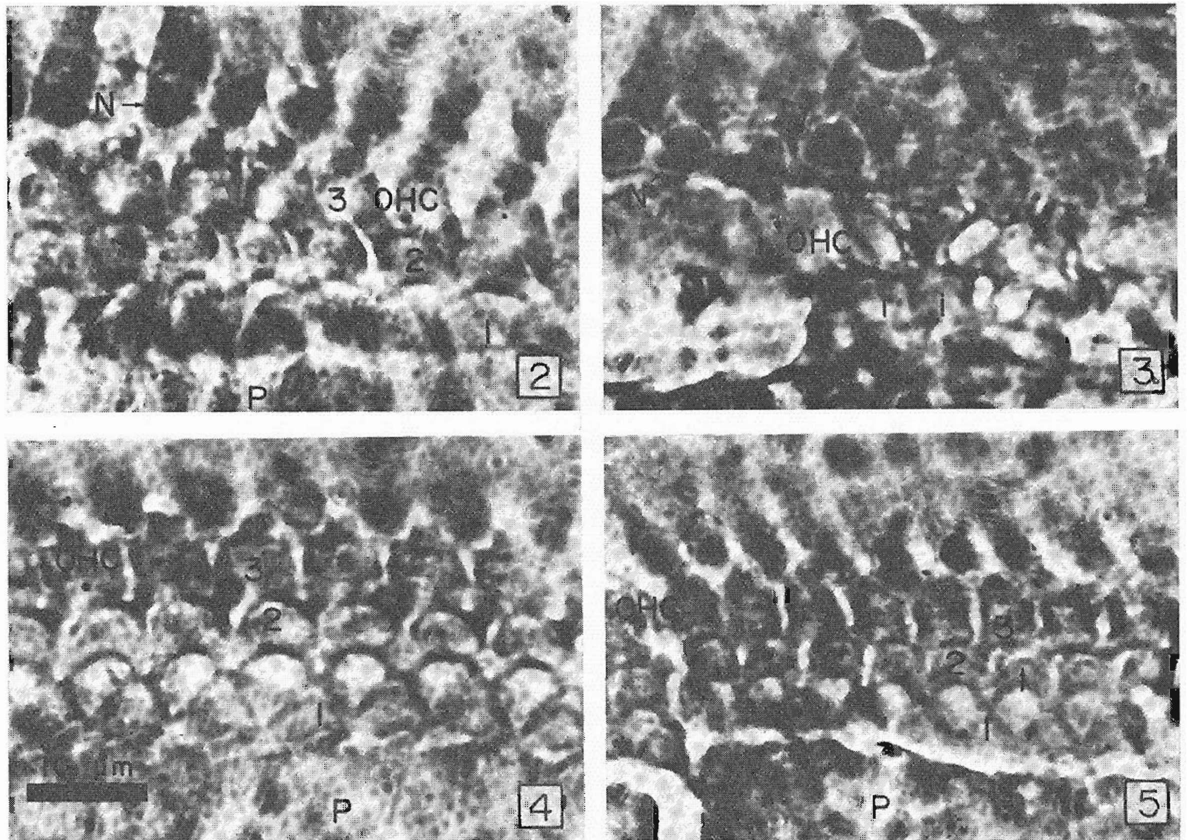


Fig. 2. Control high-line mouse, basal portion, score = 0. No pathology. 3 rows of outer hair cells (OHC 1, 2, 3) and pillar cells (P) are undamaged. Note the row of outer hair cell nuclei (N). Micrograph taken at 1200 × magnification.

Fig. 3. Primed high-line mouse, basal portion, score = 4. Massive outer hair cell damage (OHC); 3 rows can no longer be distinguished. In the center, row 1, are two outer hair cells (i) that appear normal at the level of the reticular lamina. However, inspection of outer hair cell nuclei (N) reveals that these two cells are enucleated. Only 4 nuclei remain, swollen 22% compared to control nuclei in Fig. 2. Pillar cells and inner hair cells are not visible in the micrograph but were found to be undamaged. Micrograph taken at 1200 × magnification.

Fig. 4. Control low-line mouse, basal portion, score = 0. No pathology. 3 rows of outer hair cells (OHC 1, 2, 3) are undamaged. Micrograph taken at 1200 × magnification.

Fig. 5. Primed low-line mouse, basal portion, score = 0. Low-line mice possess outer hair cells (OHC 1, 2, 3) resistant to noise-induced damage. Note stereocilia (arrow) visible on most row 2 outer hair cells. Micrograph taken at 1200 × magnification.

the reticular lamina (row 1, center), but are seen to lack nuclei. Only 4 outer hair cell nuclei are present, and these are edematous, swollen some 22% more than corresponding nuclei in Fig. 2. Fig. 4 is taken from an unprimed but retested low-line mouse (score = 0). There is no damage. Fig. 5 is taken from a primed and retested low-line mouse (score = 0). There is no evidence of any pathology. Three rows of outer hair cells are clearly visible, and stereocilia are within focus on many of them.

Effects of kanamycin treatment and retest on the cochlea

Kanamycin treatment produced a pattern of cochlear damage in all 3 lines

TABLE IV

Effects of kanamycin on cochlear hair cells

N refers to the number of cochleae examined. Per cent damage refers to the ratio of missing or damaged hair cells to total hair cells observed. On the average between 50 and 350 outer hair cells were observed per section.

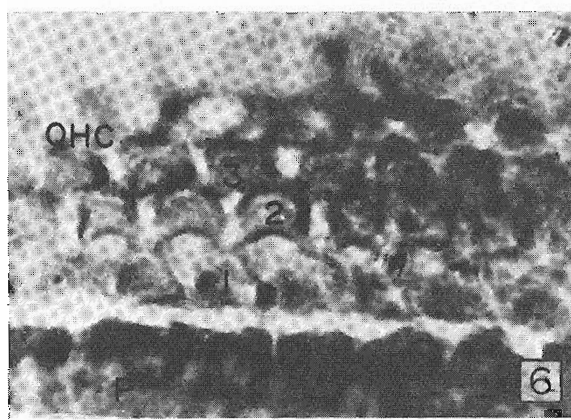
Selected line	*Portion of cochlea*	*Per cent outer hair cell damage*			
		*Controls**		*312.5 mg/kg kanamycin*	
		N	*% Damage*	*N*	*% Damage*
High	Basal portion	6	0	7	83
High	Middle portion	6	0	3	77
High	Apical portion	3	0	4	0
Unselected	Basal portion	4	1	3	93
Unselected	Middle portion	3	0	3	68
Unselected	Apical portion	4	0	4	0
Low	Basal portion	4	0	5	33
Low	Middle portion	4	0	5	37
Low	Apical portion	8	0	3	0

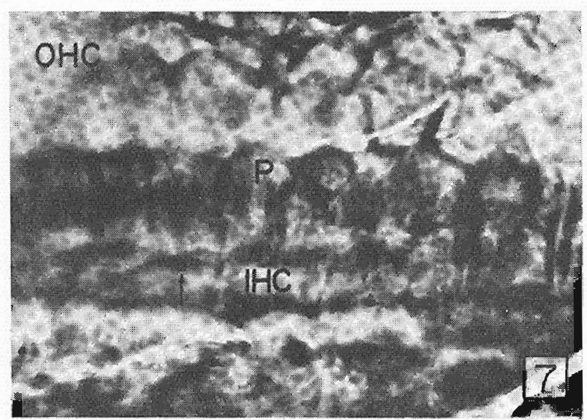
* Seizure severity score = 0.

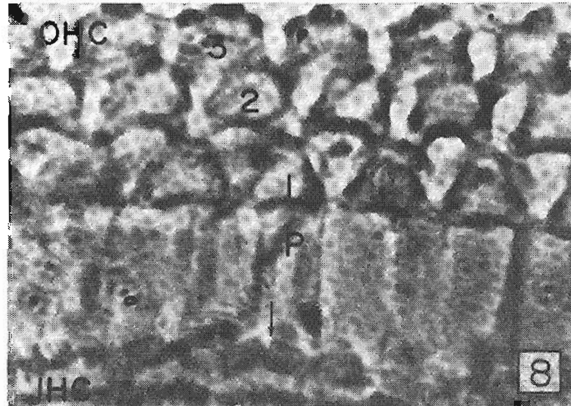
qualitatively similar to that observed in high and unselected line animals after acoustic priming. These data are summarized in Table IV.

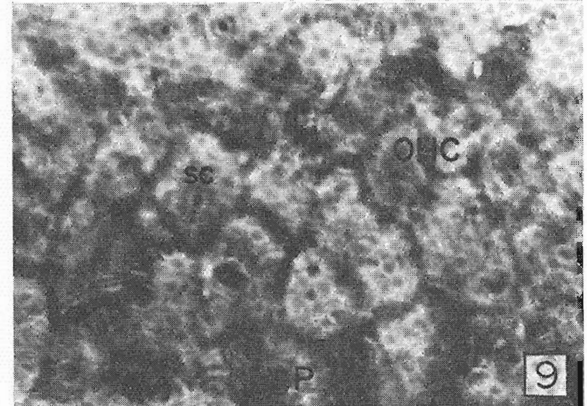
In animals of the high and unselected lines, kanamycin produced massive, almost total destruction of outer hair cells along the basal 1/3 of the cochlea. The damage was significantly less severe in the basal cochlea of low-line mice where only about 33% of the outer hair cells were missing or damaged. Per cent damage decreased slightly along the middle 1/3 of the cochlea in animals of the high and unselected lines and remained approximately the same for animals of the low line. The apical portion of cochleae from all 3 lines remained undamaged after kanamycin treatment. Damage to pillar cells was only occasionally observed; most often this consisted of missing outer pillar cells and was usually accompanied by a tear in the organ of corti above the tunnel of corti. Even where outer hair cell destruction was complete, inner hair cells generally remained intact with normal stereocilia. Representative photomicrographs of the basal cochlea from kanamycin-treated animals and their respective controls are shown in Figs. 6–11.

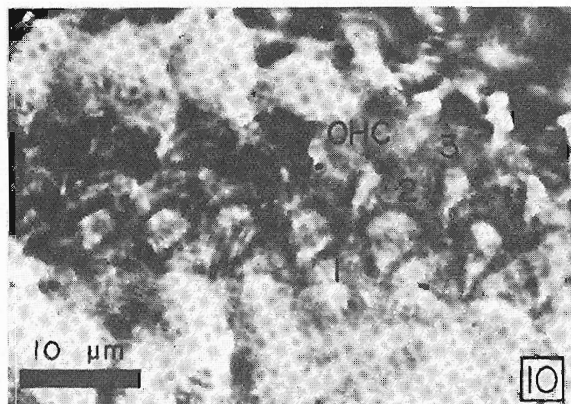
Fig. 6 is taken from a control, high-line mouse retested at 26 days of age (score = 0). The heads of the outer pillar cells are seen along with 3 rows of outer hair cells and no pathology is evident. The pattern of stereocilia of the outer hair cells in row 2 exhibits the wide open configuration typical of the basal cochlea. Fig. 7 shows a kanamycin-treated high-line mouse retested at 26 days of age (score = 4). The 3 rows of outer hair cells are totally degenerated, leaving behind only cellular debris and an irregular pattern of fibrous structures, the remnants of outer hair cell membranes. In contrast to the outer hair cell damage, the pillar cells are undamaged. Inner hair cells are also unaffected. Note the stereocilia visible on the inner hair cells at the left center











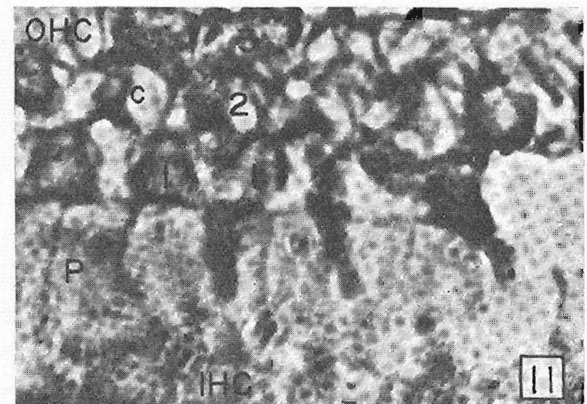


Fig. 6. Control high-line mouse, basal portion, score = 0. No pathology. 3 rows of outer hair cells (OHC 1, 2, 3), and heads of outer pillar cells (P) are visible. Micrograph taken at 1200 × magnification.

Fig. 7. Kanamycin-treated high-line mouse, basal portion, score = 4. Complete destruction of outer hair cells (OHC); 3 rows can no longer be distinguished. In sharp contrast to the condition of the outer hair cells the pillar cells (P) and inner hair cells (IHC) are undamaged. Note normal stereocilia (arrow) on inner hair cells. Micrograph taken at 1200 × magnification.

Fig. 8. Control unselected-line mouse, basal portion, score = 0. No pathology. 3 rows of outer hair cells (OHC 1, 2, 3), pillar cells (P) and inner hair cells (IHC) are visible. Note stereocilia on inner hair cells (arrow). Outer hair cell stereocilia are out of the focal plane. Micrograph taken at 1200 × magnification.

Fig. 9. Kanamycin-treated unselected-line mouse, basal portion, score = 2. Complete destruction of outer hair cells (OHC). Three rows can no longer be distinguished. Only phalangeal scars (sc) remain. Pillar cells (P) appear normal. Micrograph taken at 1200 × magnification.

Fig. 10. Control low-line mouse, basal portion, score = 0. No pathology, 3 rows of outer hair cells (OHC 1, 2, 3) are seen. Undamaged pillar cells and inner hair cells are out of the focal plane. Micrograph taken at 1200 × magnification.

Fig. 11. Kanamycin-treated low-line mouse, basal portion, score = 0. Severe damage to all 3 outer hair cell rows (OHC 1, 2, 3) although not as complete as that seen in Figs. 3 and 5. Some outer hair cells remain intact (i) and others are in the process of cytoplasmic shrinkage (c). Pillars (P) and inner hair cells (IHC) are undamaged. Micrograph taken at 1200 × magnification.

of the micrograph. Damage to stereocilia is typically the first pathological sign seen after damage to hair cells[8]. Fig. 8 is taken from a control unselected-line mouse, retested at 26 days of age (score = 0). The regular pattern of the undamaged organ of corti is evident. Inner hair cells at the bottom of the micrograph exhibit stereocilia. Fig. 9 is from a kanamycin-treated unselected-line mouse retested at 26 days of age (score = 2). Outer hair cell damage is complete and all that remains is a regular pattern of phalangeal scarring. The heads of the outer pillars are visible and are seen to be undamaged. The inner hair cells (not visible in the micrograph) were also found to be unaffected. There is a tear in the organ of corti at the lower left in the micrograph, near the junction of the outer hair cells and the outer pillar cells. Such tears were occasionally observed in basal cochlear specimens from kanamycin-treated animals but were never seen in control animals. Fig. 10 is taken from a control low-line mouse retested at 26 days of age (score = 0). There is no pathology of the outer hair cells, pillar cells or inner hair cells. The latter are out of the plane of focus of the micrograph. Fig. 11 is taken from a kanamycin-treated low-line mouse retested at 26 days of age (score = 2). There is massive outer hair cell damage, although not as complete as that seen in identically treated high- and unselected-line mice. Outer hair cells are seen in varying stages of degeneration, from intact cells (left-most 2 cells in row 1) to completely degenerated cells (rows 2 and 3, upper right). The left-most outer hair cell in row 2 is a good example of the cytoplasmic shrinkage with the cell membrane remaining intact, attached via desmosomes to the reticular lamina seen in degenerating outer hair cells described by Engstrom et al.[8]. Pillar cells appear normal, and undamaged inner hair cells (just barely in the field of focus) are seen at the extreme bottom of the micrograph.

DISCUSSION

In a previous study Deckard et al. have reported the results of a selective breeding experiment in which mice were bred for acoustic priming efficacy[7]. The breeding experiment was successful, and lines of animals which were either susceptible or resistant to acoustic priming were developed; and an unselected control line was also maintained. In the experiments reported here, mice from the 17th, 18th and 19th generations of this selective breeding experiment were used. Acoustic priming at 19 days of age was sufficient to induce subsequent audiogenic seizure susceptibility in animals from the high and unselected lines, but not in mice of the low line. Histological examination of the cochleae of these animals showed that mice of the high and unselected lines suffered large-scale outer hair cell damage at the basal end of the cochlea. Inner hair cells were undamaged. Control animals of all 3 lines, that is, animals not exposed to the priming stimulus, and primed animals of the low line, all of which failed to exhibit audiogenic seizures upon retest, exhibited no cochlear pathology.

These data suggest that in selectively breeding mice for acoustic priming efficacy, Deckard et al.[7] in fact selected in part for mice which differ in the degree to which intense auditory stimulation causes hair cell damage. This hypothesis is reinforced by

the observation that in those rare cases of primed and retested low-line animals which suffered audiogenic seizures, histological examination of the cochleae revealed hair cell damage. In these cases, a pattern of outer hair cell damage similar to that seen in primed and retested seizure-susceptible mice of the other two lines was observed.

Chronic administration of kanamycin caused the development of audiogenic seizure susceptibility in mice of all 3 lines. Histological examination of the cochleae of these animals revealed extensive damage to the outer hair cell population in the basal portion of the cochlea, the inner hair cells remaining unaffected. Thus, it appears that outer hair cell damage of considerable extent is sufficient to cause the occurrence of subsequent audiogenic seizure susceptibility at ages ranging from 3 to 4 weeks in animals of these selected lines. These data are congruent with electrophysiological evidence which indicates that acoustic priming reduces the amplitude of the cochlear microphonic[17,19]. The cochlear microphonic is generally assumed to reflect the functioning of the outer rows of hair cells, the output from inner cells being at least 60 dB down from that produced by the former[5,6].

To explain the effects of acoustic priming on subsequent audiogenic seizure susceptibility, a number of investigators have proposed a sensory deprivation supersensitivity hypothesis[17,22,23]. This hypothesis assumes that the very loud priming stimulus damages the cochlea in some way, thereby causing a partial deprivation or disuse of afferent input to a higher, more central, auditory structure. By a presently unexplained mechanism, higher auditory structures respond to this lack of input by becoming supersensitive, so that upon re-exposure to the intense priming stimulus a massive afferent volley originating in the remaining intact cochlear elements is produced in the auditory pathway, triggering the adiogenic seizure.

This hypothesis is supported by the following observations: first, and as we have already indicated, acoustic priming reduces the amplitude of the cochlear microphonic. Most BALB/c mice which had been primed failed to exhibit any cochlear microphonic response when stimulated with a moderately loud click stimulus (60 dB at 17 kHz). In those primed animals which did show a cochlear microphonic response when stimulated, the response amplitude was only 1.1 μV compared to 90 μV for unprimed control animals[17]; second, Henry et al.[13] showed that at relatively low stimulus intensities (sounds below 65 db), primed mice had significantly smaller auditory-evoked potentials than did non-primed control animals. However, at higher stimulus intensities the relation between prior exposure to a priming stimulus and size of the auditory-evoked potential was exactly reversed, indicating that recruitment deafness had been produced in primed animals[13]. Third, acoustic deprivation, produced either by damaging the tympanic membrane or by blocking the auditory canal, increased susceptibility to audiogenic seizures. To these data we now add our observation that treatments which render animals which were previously resistant to audiogenic seizures susceptible to this mode of seizure induction cause damage to sensory receptors, that is, damages outer hair cells in the basal portions of the cochlea.

The precise mechanism and location of the development of damage/disuse supersensitivity following outer hair cell damage are as yet unknown. Dallos and Harris, in recording from auditory nerve fibers originating in an outer hair cell free

region of chinchilla cochleae found no evidence of supersensitivity in their preparations. Spontaneous rates of discharge of VIIIth nerve fibers were found to be unaltered compared to control preparations[6].

The fact that VIIIth nerve functioning is relatively normal in the absence of outer hair cells suggests that the mode of supersensitivity development need possibly not be attributed to a classical receptor proliferation at the postsynaptic site immediately following the damaged elements as is the case in denervated skeletal muscle fibers[24–26]. An alternate possibility for modification of auditory functioning exists with respect to descending centrifugal controls. One such pathway, the olivocochlear bundle (OCB) takes its origin from the retrolateral olivary cell group in the superior olivary complex and terminates directly on the inner and outer hair cells of the cochlea. Some of the fibers descending in the OCB traverse the vestibular nerve and reach the cochlear nucleus (CN). Most of these fibers terminate in the superficial granular layer of the ventral CN, while the remainder enter the nucleus itself. These cholinergic fibers have been demonstrated to exert an inhibitory effect on the auditory system[29]. It is conceivable that some type of disinhibition following peripheral damage or disuse results in increased firing to a loud acoustic stimulus.

Another body of fibers, originating in the superior olive, passes through the intermediate striae and reaches the anteroventral CN. Electrical stimulation of the medial portion of the superior olive has been shown to produce an increased discharge of neurons in the anteroventral CN to an acoustic stimulus. Furthermore, the application of weak current to the medial superior olive decreases the threshold for an auditory stimulus by at least 10 db SPL when measured by single unit recording in the ipsilateral antero-ventral CN. Iontophoretic application of acetylcholine to neurons in the anteroventral CN has exactly the same effect as electrical stimulation of the medial superior olive, i.e. a lowering of the threshold for acoustic stimulation. Upon application of cholinergic blockers, e.g. gallamine and β-erythroidine, thresholds for sound stimulation are raised, according to Whitfield 'perhaps to infinity'[29]. Thus it is possible that a hyperfunctioning of this pathway could result in increased responsiveness to acoustic stimulation.

In summary, the results of this experiment indicate that: (1) in selectively breeding animals for differential acoustic priming efficacy Deckard et al.[7] in fact selected in part for animals which differ in the degree to which loud sounds affect the cochlear receptors; and (2) treatments which produce acoustic priming damage cochlear receptors. Further research is necessary to show precisely how receptor cell damage leads to the development of supersensitivity in more central neural structures.

ACKNOWLEDGEMENTS

This research was supported in part by NIGMS Training Grant GM 07305.

We thank Richard L. Sgroi of Bristol Laboratories for kindly supplying us with kanamycin. We thank S. J. Young for performing the spectral analysis of the priming stimulus, and C. J. Wilson for a critical review of this manuscript. We also thank Deborah Commins for her skilled technical assistance.

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