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Genetic influences on early cochlear vulnerability to noise and protection from noise by low-dose kanamycin in hybrid mice

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GENETIC INFLUENCES ON EARLY COCHLEAR VULNERABILITY TO NOISE AND PROTECTION FROM NOISE BY LOW-DOSE KANAMYCIN IN HYBRID MICE

by

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***Abstract:** Experiments evaluated both noise vulnerability and the extent of protection from noise by sub-chronic low-dose kanamycin in young F1 hybrids resulting from a cross between C57BL/6J and CBA/J inbred mice.*

ACKNOWLEDGEMENTS

I would firstly like to thank Dr. Kevin Ohlemiller for his knowledge and support as a professor and throughout my capstone year. I am truly blessed to have been able to work with someone who takes so much pride in his work and students. Thank you for your patience and wealth of knowledge throughout my research experience. I have a newfound appreciation for basic science and research and I owe that mostly to my work in your lab. I would like to thank Erin Rellinger for her consistent advice and support with my project. Your guidance and friendship helped me through this process. I would also like to acknowledge Michelle Hungerford for teaching me how to obtain ABRs on mice. My project would not have happened without the aid of these great people.

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ABBREVIATIONS

ABR	Auditory Brainstem Response
B6	C57BL/6J
dB SPL	Decibels (sound pressure level)
g	grams
Hz	Hertz
JAX	Jackson Laboratory
kg	kilogram
KM	Kanamycin
kHz	Kilohertz
mg	milligram
min	minutes
ml	milliliter
NIPTS	Noise Induced Permanent Threshold Shift
sec	seconds
SD	standard deviation

INTRODUCTION

Young animals and humans alike are especially susceptible to cochlear injury due to aminoglycoside toxicity and loud noise exposure (Henry, Chole, McGinn & Frush, 1981, Henry, 1984a). An early, "critical period" has been identified in which both cause more damage than they would to a developed, adult ear (Saunders & Chen, 1982). This period of vulnerability spans a different range of time for different species, but exceeds the period required for development and initial function of the inner ear (Henry, 1984a; Henley & Rybak, 1995). It has therefore been postulated to result from slower development of protective or homeostatic functions. Here we consider the genetic foundations of the critical period as manifested in inbred mice, and how this intersects with another recently noted phenomenon, namely protection against cochlear noise injury by small amounts of aminoglycosides.

Aminoglycoside Ototoxicity

Many studies have shown ototoxic effects of aminoglycoside antibiotics (Rizzi & Hirose, 2007; Li & Steyger, 2009). These antibiotics are prescribed frequently, and can be very effective at treating certain bacterial infections. Ototoxicity related to aminoglycosides is associated with hearing loss and/or vestibular loss due to hair cell death within the endorgans themselves. Some aminoglycosides, such as gentamicin, specifically attack vestibular hair cells, while drugs like kanamycin (KM), have more of an effect on the cochlear hair cells (Selimoglu, 2007). Principal effects are typically on outer hair cells in the basal portion of the cochlea resulting in high frequency hearing loss. All hair cell loss due to aminoglycoside toxicity in mammals is permanent in nature (Hirose & Sato, 2011). Once taken into the cells, aminoglycosdes have the

ability to generate toxic levels of reactive oxygen species (ROS) which initiate hair cell death by damaging DNA, proteins, and membranes (Selimoglu, 2007; Li & Steyger, 2009).

Noise Injury

Intense noise is considered a potent stressor for young animals and has been compared with aminoglycosides for its ability to harm the developing cochlea (Selimoglu, 2007). There are a number of parallels in the manner in which these operate. Acoustic trauma can cause widespread damage throughout the cochlea, but, just as for aminoglycosides, the main damage is often to the outer hair cells. Also similar to aminoglycosides, noise causes hair cell death most readily at the basal end of the cochlea, often while leaving inner hair cells less affected (Saunders & Chen, 1982). Finally, like aminoglycosides, acoustic overstimulation can promote oxidative stress resulting in hair cell death due to apoptosis (Li & Steyger, 2009).

Interactions between Aminoglycosides and Noise Exposure

Synergistic interactions between aminoglycosides and noise have been documented. Ryan and Bone (1982) found that chinchillas that were administered kanamycin and noise simultaneously had more extensive hearing loss than those only exposed to the kanamycin alone. Brown , Brummett, Fox & Bendrick (1980) gave guinea pigs daily subcutaneous injections of kanamycin at varying amounts followed by a ten-hour noise exposure (115 or 45 dB) for seven consecutive days. They found that the combined administration of kanamycin and acoustic exposure led to greater cochlear damage than caused by either agent when given alone. This finding as well as many others (e.g., Brummett, Fox & Kempton, 1992) suggests synergistic effects.

Mouse Models of Noise and Aminoglycoside Injury

Animal models have played a crucial role in the understanding the mechanisms of hearing loss. Mouse models have proven particularly useful because they are genetically homogeneous and offer the potential for transgenic studies (Poirrier, Van den Ackerveken, Kim, Vandenbosch, Nguyen, Lefebvrea & Malgrange, 2010), as well as mapping of useful phenotypic differences. Due to rapid maturation, the cochlear critical period for both noise and ototoxicity can be studied easily in the mouse (Henry & McGinn, 1992). Hearing-related differences between two particular inbred strains (CBA/J and C57BL/6) have been discussed in the literature for decades. CBA/J mice are generally used as a model of good hearing that is particularly resistant to presbycusis (age-related hearing loss) (Fernandez, Ohlemiller, Gagnon & Clark, 2010). C57BL/6J (B6) mice, however, carry the *Cdh23*^{ahl} allele that promotes progressive hearing loss with aging. Generally, B6 mice are considerably more vulnerable to noise exposure than the same aged CBA/J strain (Henry, 1984a; Ohlemiller & Gagnon, 2007), yet may be more resistant to aminoglycoside toxicity (Wu, Sha, McLaren, Kawamoto, Raphael & Schacht, 2001). Differences in noise-induced hearing loss and ototoxicity across inbred strains may reflect differences in metabolism, cellular uptake mechanisms, or differential expression of antioxidant enzymes (Wu et al., 2001).

Heightened Noise Vulnerability in CBA/J Mice

CBA/J mice are extremely susceptible to noise-induced permanent threshold shifts (NIPTS) at a young age and become less affected as they become older. It was found that 5 minutes of 124 dB SPL broadband noise caused a significantly higher threshold shift in 20 day old mice compared to 60 and 180 day old CBA/J mice (Henry, 1992). Fernandez et al. (2010)

found that as little as 30 sec of broadband noise exposure (110 dB SPL) caused severe NIPTS and moderate hair cell loss in 30 day old CBA/J mice. A follow-up study (Ohlemiller, Rybak Rice, Rellinger & Ortmann, 2011) confirmed that 6 week old CBA/J mice depart notably from closely related CBA/CaJ mice at 6 month of age, while the two strains appear similar at 6 months.

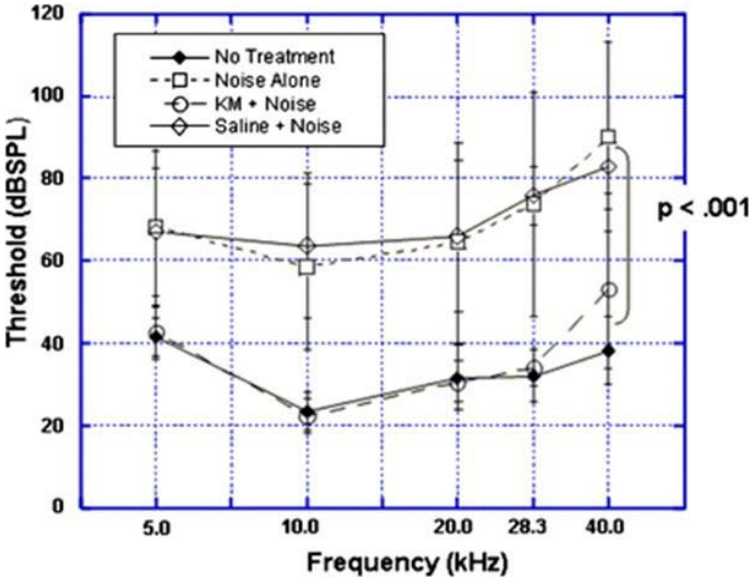

Figure 1: Mean (+SD) ABR thresholds for 30 day old CBA/J mice receiving no treatment, noise alone, KM + noise, and saline + noise.

Reprinted with permission from Fernandez et al., 2010

Previous Studies Leading to the Present Question

Despite a preponderance of evidence supporting synergy of cochlear injury by combined noise and ototoxic exposure, it was recently demonstrated by Fernandez et al. (2010) (Figure 1), that sub-chronic low-dose kanamycin (KM, 300 mg/kg sc, 2x/day, 10 days) followed by 30 seconds of high intensity (110 dB SPL) broadband noise dramatically reduced NIPTS and subsequent hair cell loss in young CBA/J mice compared to control mice receiving saline and noise. In fact, for the conditions applied, kanamycin completely protected the animals' hearing as measured by ABR. It was suggested that sub-chronic, sub-toxic exposure to kanamycin elicits a form of 'preconditioning', a well-established process whereby exposure to a mildly injurious stressor affords protection against a later, potentially permanently injurious, stressor (Gagnon, Simmons, Bao, Lei, Ortmann & Ohlemiller, 2007). Apparent preconditioning of the cochlea by

KM joins several noxious treatments shown to confer protection against cochlear noise injury. These include hypoxia (Gagnon et al., 2007), hyperthermia (Paz, Freeman, Horowitz & Sohmer, 2004), and noise (Canlon & Fransson, 1995).

Mechanistic studies of exactly how low-dose KM protects the cochlea first required studies that better define the requirements of protection with regard to dosing, age of treatment, and genetic background. Rybak Rice (2009) conducted a follow-up study using CBA/J mice of the same age and type of exposure as in the Fernandez et al (2010) study to determine the minimal dosing frequency of KM needed to protect from NIPTS. Mice were injected with KM once daily, once every other day, or once every third. Results indicated all treatment groups showed protection from NIPTS. This study also examined the possibility of KM alone causing injury, even as it rendered protection. A once daily dose of KM with no noise exposure was given for 10 days and final ABR testing showed no hearing loss due to KM alone.

Rosen (2010) conducted several experiments to test the generality of KM preconditioning. The first series sought to identify the minimal effective dosing regimen that conferred full protection against noise. Young CBA/J and B6 mice were given either a single dose (24 and 48 hours pre-noise exposure), or two doses over 3 days at 72, and 24 hours prior to exposure. It was concluded that more than two KM applications every 72 hours are required to achieve full protection. Rosen (2010) also tested the KM paradigm in older CBA/J mice (2 months of age) to see if protection was related to the critical period for ototoxicity (≤1 mo in mice). Finally, Rosen (2010) tested genetic background effects on protection by KM and found no protection in the B6 mice from the same KM exposure that impressively protected the CBA/J mice. A subsequent study (Ohlemiller, Rybak Rice, Rosen, Montgomery & Gagnon, in press) confirmed a complete lack of protection—and even possible direct injury—by KM in B6 mice

versus striking protection in CBA/J (Figure 2). Such strain differences indicate that B6 mice probably carry alleles that either promote net injury by KM or impair the engagement of protective cascades.

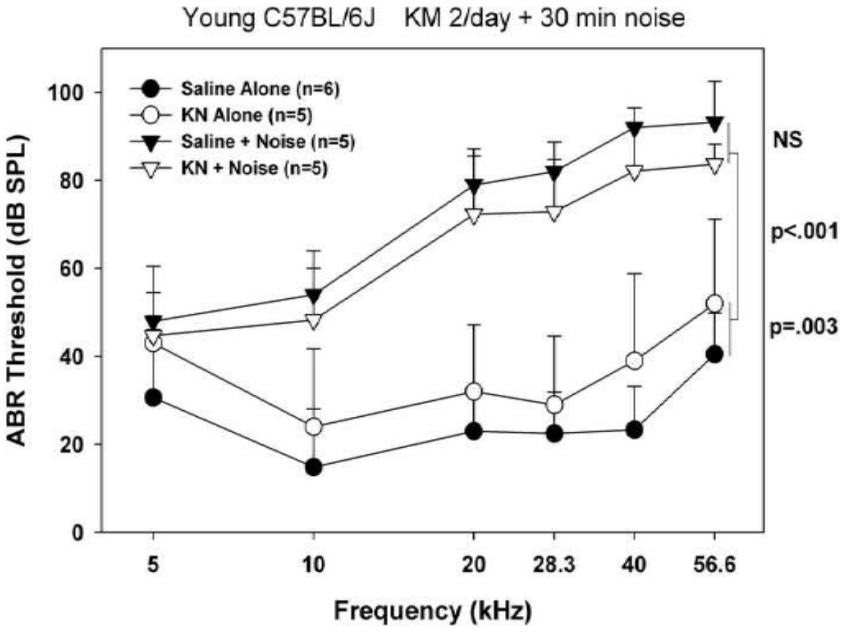

Figure 2: Mean (+SD) ABR thresholds in 6 week old C57BL/6J mice receiving KM or saline prior to 30 min noise exposure.

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Purpose of the Present Study

As summarized above, young CBA/J and B6 mice exhibit strikingly different hearing related phenotypes in two regards. First, CBA/J mice are robustly protected against cochlear noise injury by small amounts of KM, while B6 appear either unaided, or harmed. Second, at 1-2 months of age, CBA/J mice appear far more vulnerable to noise, in that the 'threshold' duration for NIPTS is roughly 1.0 minute in CBA/J versus about 3.0-4.0 minutes in B6. These differences suggest that the two strains harbor different alleles at unknown genetic loci that determine completely novel traits—early noise vulnerability and kanamycin 'protectability'. The standard approach to genetic analysis of such traits is to generate F1 hybrid mice from the two strains possessing the different phenotypes of interest. If few, highly penetrant, genes are involved, the phenotype of the F1 mice will strongly resemble one of the parent strains, defining that strain as possessing the dominant phenotype. If F1 mice essentially recapitulate the phenotype of one parental strain, the standard next step toward preliminary mapping of the genes

involved is to backcross the F1 mice to the recessive parental strain and then correlate phenotypes with genomic markers.

We posit that young CBA/J and B6 mice carry different alleles at a small number of gene loci that are separately responsible for their different responses to kanamycin and different sensitivities to noise. Accordingly, the purpose of this study was to evaluate both noise vulnerability and the extent of protection from noise by low-dose kanamycin in 6 week old F1 hybrids resulting from a cross between CBA/J and B6 mice. As we show, the F1 mice reveal a susceptibility to noise and 'protectability' by KM that largely recapulate the characteristics of CBA/J mice (Ohlemiller, Rosen & Gagnon, 2010). That is, CBA/J alleles appear semi-dominant over B6 alleles in each case. These results lay the foundation for preliminary mapping and ultimate identification of the genes underlying novel hearing phenotypes.

MATERIALS AND METHODS

Overall Strategy

Our experiments consisted of two largely non-overlapping projects. To establish the noise vulnerability of the hybrid mice required the construction of a noise duration dose-response curve, whereby both the probability and extent of NIPTS were assessed for each strain as the duration of a single noise exposure was varied. By constructing this relation in 6 wk old F1 mice, it was then possible to compare the data with similar analyses in CBA/J and B6 mice at the same age. To establish the KM 'protectability' of the hybrid mice, it was necessary to expose these to noise of a duration known to inflict moderate NIPTS on both CBA/J and B6 mice. The goal was for the F1s to sustain clear NIPTS that could either be exacerbated or reduced by KM treatment. This design required 6 experimental groups [3 strains (B6, CBA/J, F1) x 2 treatments (KM, saline)].

Animals

The study utilized 16 CBA/J, 18 C57BL/6 (B6), and 45 [B6 x CBA/J] F1 hybrid mice. All experimental groups included mice of both genders. Mice were bred from in-house breeding pairs that were purchased or derived from breeders purchased from The Jackson Laboratory (JAX). All mice were housed in the Central Institute for the Deaf Animal Colony. During treatment and recordings the mice were housed in the Mechanisms of Cochlear Injury Laboratory at Washington University School of Medicine where they had access to food and water. Approximately half of the F1 hybrid mice were used to determine noise vulnerability and the remaining mice were all used in the kanamycin/saline paradigm. All procedures were approved by the Animal Studies Committee at Washington University School of Medicine.

Noise Exposure

Noise exposures were performed in a foam-lined, single-walled soundproof room (Industrial Acoustics, Bronx, NY). One or two mice were placed inside a 21 x 21 x 11 cm wire cage that was mounted on a B&K 3921 turntable. The cage was suspended between four speakers at 0, 90, 180, and 270 degrees azimuth. Broadband noise between 4 and 45 kHz at 110 dB SPL was produced and filtered with General Radio 1310 generators and Krohn-Hite 3550 filters. The cage was rotated at approximately 0.013 Hz throughout the duration of the exposure to assure uniform exposure to each animal throughout the presentation of stimuli.

For experiments examining overall noise vulnerability of F1s, exposures were varied by multiples of two between 0.23 minutes and 3.75 minutes. The noise duration for the

kanamycin/saline study was 30 minutes across mouse strains. This duration was chosen based on previous work (Ohlemiller et al., 2000).

Auditory Brainstem Response recordings

Audiometric data were determined from evoked ABRs, a noninvasive measure of cochlear function. ABR testing, like, noise exposures were carried out in a double-walled soundproof booth (IAC). Mice were anesthetized with an intramuscular injection of 80 mg/kg ketamine/5 mg/kg xylazine solution. They were then positioned dorsally in a custom headholder with their right ear 7 cm from the sound source. Body temperature was maintained at 37.5±1°C with the use of a controlled heating pad and a rectal probe. Platinum needle electrodes were inserted subdermally behind the right ear (active), at the vertex (reference), and in the back (ground). The left ear was clamped shut to ensure it was not responding to the stimuli and recorded thresholds were only from the right ear.

The ABR threshold at each test frequency was defined as the lowest stimulus level at which Wave I was clearly present using a 5 dB step size. Wave I was used because it is the most robust wave of the mouse ABR, and thought to be generated entirely by the cochlear auditory nerve activity (Zheng, Johnson & Erway, 1999). All thresholds were verified by repetition at each frequency tested. Stimuli were 5 ms tonebursts presented at 1,000 repetitions at 5, 10, 20, 28.3, 40, and 56.6 kHz. Stimulus presentation and data acquisition used Tucker Davis Technologies System II hardware and Biosig 32 software. A two-way analysis of variance (ANOVA) followed by posthoc Bonferroni multiple comparisons tests were applied to test for significant ABR threshold differences by experimental group and frequency. Statistics were performed using SigmaStat software.

F1 Noise Vulnerability

Procedures for this aspect of the study involved a baseline hearing assessment, broadband noise exposure, a second hearing assessment, and sacrifice for histology. Mice were divided into groups of varying noise durations (see Table 1). Baseline auditory brainstem responses (ABRs) were obtained between 4 weeks post-gestational age and at least 2 days before noise exposure. Noise exposures were performed at 6 weeks post-gestational age at specified durations. A final ABR was performed at 8 weeks post-gestational age (2 weeks post noise exposure) to ensure changes in hearing were permanent threshold shifts. Once vulnerability trends were observed, mice were added for durations that bracketed the dynamic portion of the noise dose-response. In accordance with Ohlemiller et al. (2000), NIPTS was defined as an increase in threshold of at least 10 dB at 2 or more test frequencies. To permit dose-response comparisons between F1 mice and earlier estimates in CBA/J and B6 mice, data were rendered as *probability of NIPTS* versus noise duration and *total amount of NIPTS* versus duration. To compare the amount of NIPTS by group and exposure duration, average threshold shifts across frequencies (excluding 56.6 kHz) were added (Ohlemiller et al., 2011).

Table 1: Number of F1 mice per noise exposure group

Noise Duration in minutes	Number of Animals
0.23	4
0.47	8
0.94	8
1.88	4
3.75	4

Kanamycin Preconditioning

The mice that received kanamycin were injected subcutaneously twice a day for ten days with a 300 mg/kg solution (63.93 mg/mL concentration). Injections began between 29 and 30 days post-gestational age and were stopped at either 38 or 39 days post-gestational age. Mice were weighed prior to each injection and received 0.006 cc of the KM/saline solution for every gram of body weight. Injections occurred approximately every twelve hours to maintain a chronic drug state. Age and gender matched controls received the same dosage of saline subcutaneously in the same amount and at the same time interval as the kanamycin treated mice. The drug regimen was well-tolerated as no mice were lost during the course of treatment.

All mice in this aspect of the study received a baseline ABR one day prior to the onset of the injection regimen. Injections lasted a total of 10 days. A second ABR was performed on the tenth day of injections to observe if there was any effect of injections alone on hearing. Noise exposures were completed the day following final injections. Final ABRs were completed two weeks after noise exposure. Animals were then sacrificed for histology.

Table 2: Number of mice per treatment group

Mouse Strain	Treatment	Number of Animals
F1 (CBA/J x B6)	Kanamycin	9
F1 (CBA/J x B6)	Saline	8
CBA/J	Kanamycin	8
CBA/J	Saline	8
B6	Kanamycin	9
B6	Saline	9

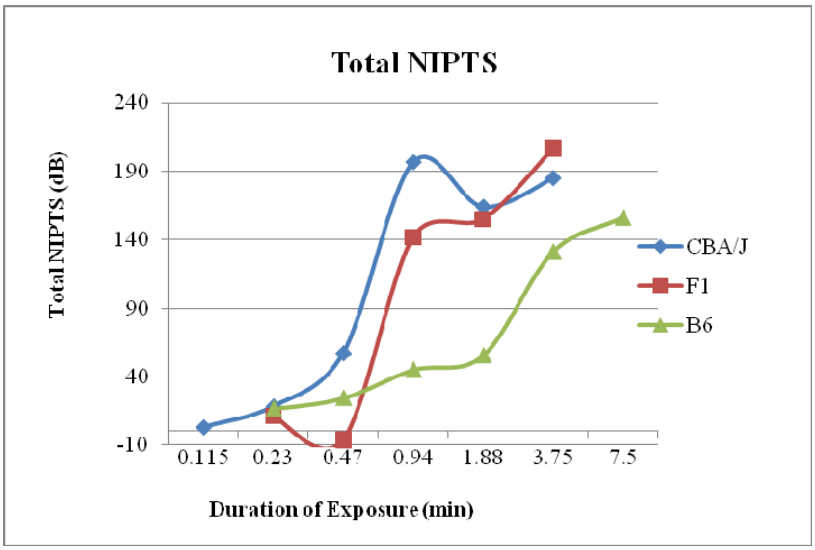
Sacrifice and tissue processing for histology

Cochleae were taken to preserve the potential for histologic evaluation (not shown here). After final ABR threshold were obtained, mice were overdosed using an intraperitoneal injection of sodium pentobarbital at 4 times the surgical dose (240 mg/kg). When no muscular response to stimulation could be elicited, mice were transcardially perfused with cold 2.0% paraformaldehyde/2.0% glutaraledehyde solution in 0.1 M phosphate buffer. Both cochleae from each mouse were quickly harvested and immersed in the same fixative, and extraneous tissue and the stapes were removed. All middle ears were inspected for signs of otitis media. No animals in this study presented with the middle ear pathology.

RESULTS

F1 Noise Vulnerability

Figure 3 shows that F1 mice were fairly similar to CBA/J in noise vulnerability, as indicated by both the total NIPTS (Figure 3A), and probability of NIPTS (Figure 3B). Figure 3A is represents the overall growth in NIPTS by strain and with exposure duration, based on the sum of the threshold shifts across frequencies (Total NIPTS in dB). For F1s, threshold shifts began to accumulate rapidly between 0.47 and 0.94 min, which is similar to published data for CBA/Js (Ohlemiller et al, 2011). B6 mice, however, required at least 3.75 min for similar NIPTS. Figure 3B compares the growth of the probability of NIPTS across strains. Like Figure 3A, Figure 3B shows that the F1 mice respond similarly to the CBA/Js to noise exposure as seen in similar threshold shifts. These results suggest that the noise vulnerability of young F1 mice, while not exactly that of CBA/J far more strongly resembles CBA/J than B6. Thus CBA/J alleles for this trait appear semi-dominant to those in B6.



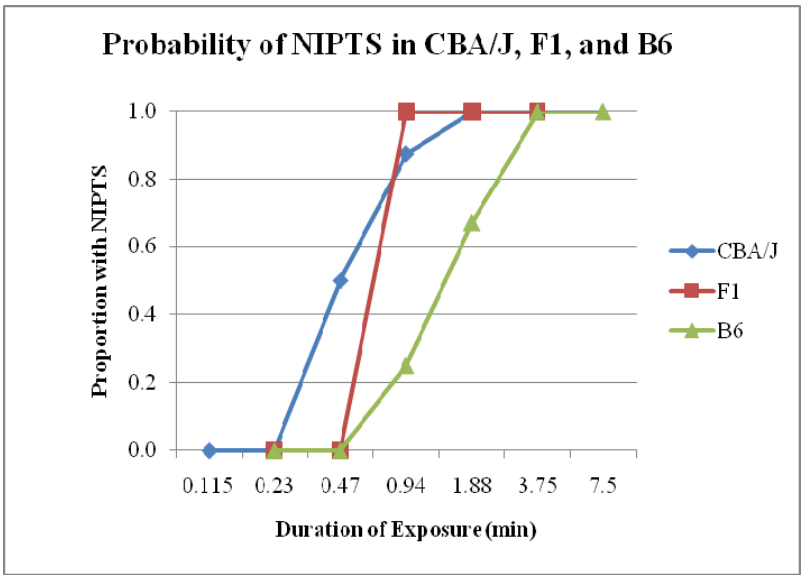


Figure 3: (A) The sum of the proportion of noise induced positive threshold shift (NIPTS) in CBA/J, F1, and C57BL/6J (B6) mice following noise exposure to 110 dB SPL broadband noise at varying durations. (B) The probability of NIPTS by strain.

CBA/J and B6 data replotted with permission from Ohlemiller et al., in press, &Ortmann et al., 2004 respectively

Kanamycin Preconditioning

As shown in Figure 4A, thresholds in both the saline and KM treated mice after noise exposure were significantly different than all other conditions ($p<0.001$), yet ABR thresholds post-noise for saline were also significantly different from those in KM treated mice ($p<0.001$). CBA/J mice receiving KM did not exhibit full protection from NIPTS, but did show significant protection, especially in the lower frequencies (5-10 kHz) tested. A potential decrease in the

efficacy of KM versus previous work may reflect the much longer duration of noise exposure in these mice (30 min versus 30 sec).

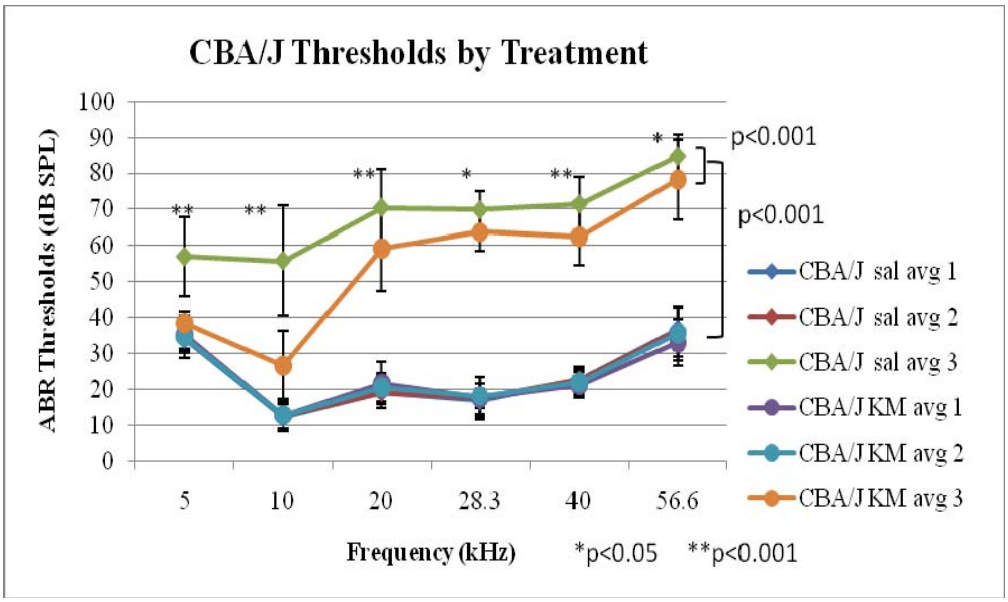


Figure 4(A): Mean (+SD) ABR thresholds for saline and KM treated CBA/J mice. The first ABR was before treatment, second after injections, and third 2 weeks after 30 min noise exposure.

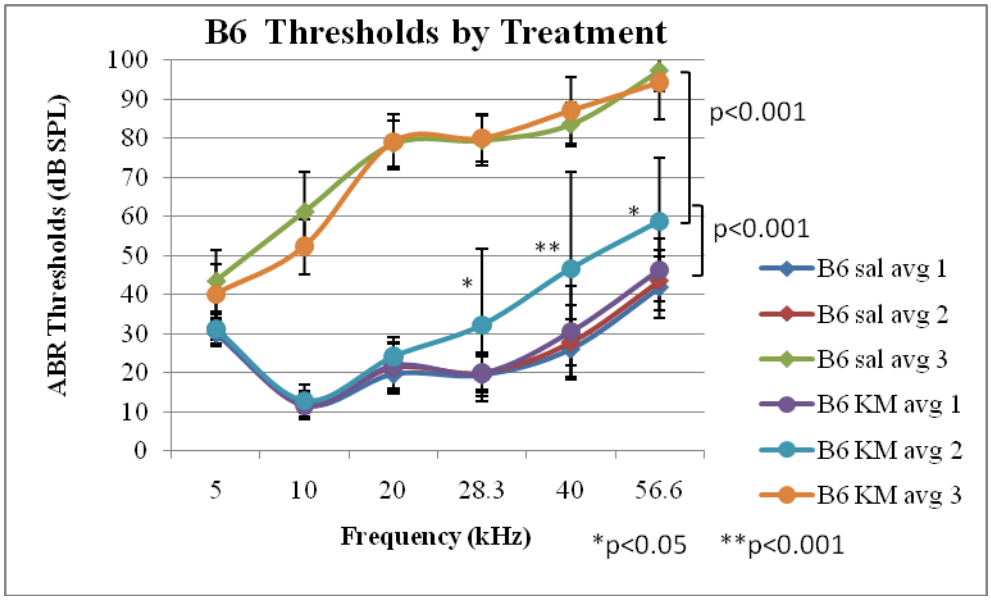


Figure 3(B): Mean (+SD) ABR thresholds for saline and KM treated C57BL/6J (B6) mice. The first ABR was before treatment, second after injections, and third 2 weeks after 30 min noise exposure.

Consistent with previous work, there appeared to be no protection from noise by KM in the B6 mice. Threshold shifts in these mice were not statistically different from mice treated with saline after noise exposure ($p<0.001$). Moreover, significant ($p<0.001$) damage also appeared to be inflicted by KM, as reflected in a high frequency hearing loss in the second ABR thresholds obtained from animals in the KM group specifically the KM average 2 (Figure 4B).

Figure 4C shows that young F1 hybrids were partially but significantly ($p<0.001$) protected from noise by KM compared to those receiving saline. Since the F1 phenotype is intermediate between that in CBA/J and B6, these findings suggest that 'protectability' is a semi-dominant trait in these two strains. Notably, F1 mice also did not appear harmed by KM when administered alone. This is seen as the absence of any difference in thresholds between the first and second ABRs compared to the significant difference between ABR thresholds in the B6 mice.

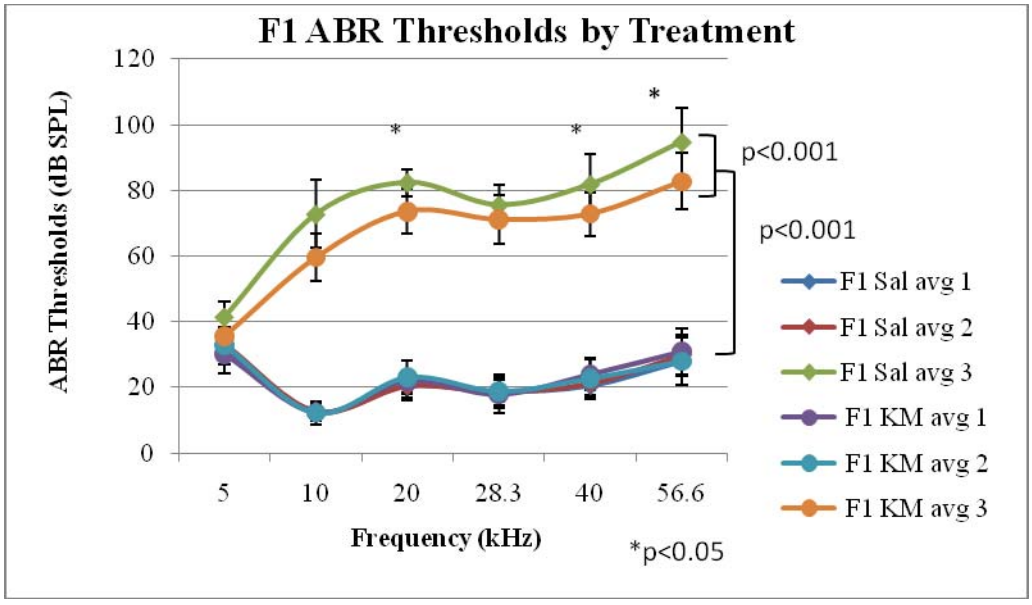


Figure 4 (C): Mean (+SD) ABR thresholds for saline and KM treated F1 mice. The first ABR was before treatment, second after injections, and third 2 weeks after 30 min noise exposure.

DISCUSSION

The purpose of this study was to evaluate both noise vulnerability and the extent of protection from noise by low-dose kanamycin in 6 week old F1 hybrids. The data show F1 susceptibility to noise and 'protectability' against noise is similar to that of CBA/J mice. While the CBA/J phenotype was slightly altered, the phenotype of the F1s was far more like that in CBA/J than B6. This suggests that young CBA/J and B6 mice carry different alleles at a potentially small number of gene loci that are separately responsible for their different responses to kanamycin and different sensitivities to noise exposure, and that CBA/J alleles are semi-dominant. While the number and type of genes remains unknown, these are worth pursuing as they establish completely novel phenotypes with probable relevance to human hearing: Heightened noise vulnerability early in life and the balance of protection versus injury caused by environmental events.

Preconditioning from Kanamycin

As shown previously and confirmed here, young CBA/J and B6 mice exhibit different effects from preconditioning due to KM. CBA/J mice are significantly protected against cochlear noise injury by low-dose, sub-chronic KM, while B6 appear either unaided, or harmed. This study probed the limits of 'protectability' in the CBA/J mice by providing 60 times more noise than used in previous work (Fernandez et al, 2010). This may explain why the extent of protection in both CBA/Js and the F1s was somewhat modest. The present data study confirms apparent 'non-protectability' of B6 mice by KM; however, this must remain tentative until more dosing levels and schedules are tested. It may be the case that apparent injury by KM and non-protectability in B6 are actually two separate phenotypes, mechanistically unrelated. Arguing

against this is the fact that both features became CBA/J-like in the F1 mice. That is, both features co-varied. This suggests that the lack of protection in B6 reflects two linked processes, protection versus injury. Since preconditioning is based on modest injury engaging innate protections, the same cascade that protects CBA/J mice may 'swing' toward injury in B6.

Noise Vulnerability

The CBA/J and B6 strains are very differently vulnerable to noise. It has been shown that CBA/J mice appear approximately 3 times more vulnerable to noise, in that the 'threshold' duration for NIHL is roughly 1.0 minute in CBA/J versus about 3.0 minutes in the B6. Our study found that the F1 hybrid mice had a more similar NIPTS from differing durations of noise to the CBA/J parent strain. These findings show a semi-dominant inheritance pattern for noise vulnerability in the F1 hybrid mice.

Implications for Genes Involved

This study explored two characteristics, basic noise vulnerability and KM 'protectability.' These traits markedly differ between CBA/J and B6 mice and likely reflect distinct sets of genes. Young CBA/J mice are much more vulnerable to noise than are B6 mice, while KM confers protection from noise exposure in the CBA/J, but not B6. F1 hybrid mice formed from these strains resemble the CBA/J mice in both regards, although not fully. It may therefore be posited that polymorphisms at a small number of loci differentiate these strains. The genes that shape these traits may impact the way environmental noise and ototoxins interact to affect the hearing of animals and humans. Natural candidates for preconditioning mechanisms that affect these genes include those known to be activated by ototoxins and other stressors, including heatshock

protein HSP70 which mediates protection by elevation of body temperature (Yoshida, Kristiansen & Liberman, 1999), hypoxic preconditioning (Gagnon et al., 2007) suggested a role for hypoxia-induced factor HIF-1α , and finally antioxidants, tumor necrosis factor TNFα, nuclear factor NFκB, and heme oxygenase HO-1 (Gidday, 2006; Rybak, 2007), all of which participate in cochlear responses to stress (Rybak, 2007).

Next Steps

Appropriate future research should take a two-pronged approach. The first of these is classical genetic, by backcrossing the F1 hybrid mice to the most recessive strain for the vulnerability and susceptibility, in this case B6. A single-gene dominantly inherited trait from CBA/J should be seen in approximately half of N2 backcross mice, according to simple Mendelian principles. Even if two or three genes are involved, any clear modes in threshold distributions would point to populations bearing particular alleles. The underlying genes could then be preliminarily mapped using genetic markers. The second method is molecular: KM and or noise should produce different patterns of gene expression in CBA/J and B6. Gene expression analysis using microarrays is increasingly commonplace. The two methods may ideally point to some of the same genes and pathways.

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Appendix A:

ABR / CAP Data Log

sheet version date 8/3/04

ID Number _____

Animal type: mouse / rat / gerbil

Strain: **Genotype** (tentative / confirmed): **Sac Date** _____

Sex: **DOB:** **Age:**

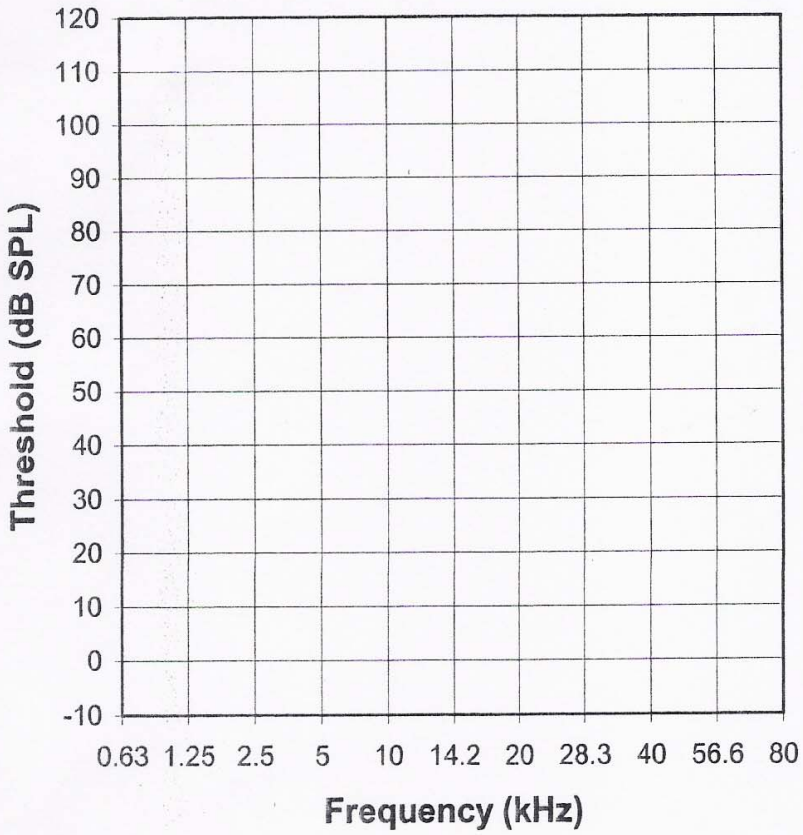
Identifying marks: L R

Project:

Collaborating PI:

ABR Conditions: 5 ms tone, 1000 reps, 20/sec, 100-10,000 Hz, x100,000, D/A 30 kHz
Anesthetic: 80/15 mg/kg (Ket/Xyl) / other: **Rise Time:** 1.0 ms / other:
Ear: Right / Left **Speaker Distance:** 7 cm / 10 cm
Ground: Back/Other: **Reference:** Vertex/Other:

CAP Conditions: 5 ms tone, 100 reps, 3/sec, 30-3,000 Hz, x100,000, D/A 30 kHz
Anesthetic: Pentobarb (60 mg/kg) / other: **Rise Time:** 1.0 ms / other:
Round window of: Right / Left **Speaker Distance:** 7 cm / 10 cm
Ground: Hindleg / Other: **Reference:** Neck musculature/Other:



Date / Time: _____
Tester (Initials):
Condition:

Date / Time: _____
Tester (Initials):
Condition:

Date / Time: _____
Tester (Initials):
Condition:

Date / Time: _____
Tester (Initials):
Condition:

Notes:

Appendix B:

Date: AM

Ear notch	Animal ID	Treatment	BW (g) (x 0.006)	Injection Volume (µL)
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( )				
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( )				
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Date: PM

Ear notch	Animal ID	Treatment	BW (g) (x 0.006)	Injection Volume (µL)
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