Expanding our view of ototoxicity as we expand our definition of hearing loss

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Thanks to those with whom I've had the good fortune to work

Northeast Ohio Medical University — Jianxin Bao, PhD

University at Buffalo, The State University of New York — Bo Hua Hu, PhD

Nationwide Children's — Kathrin Meyer, PhD

The Ohio State University

Former Ph.D. students:

- **Ryan Harrison, PhD, Ohio Department of Health**
- **Riley DeBacker, AuD, PhD, NCRAR**
- **Jason Riggs, AuD, PhD, Decibel Therapeutics**

Colleagues in Speech and Hearing Science

Colleagues in Otolaryngology

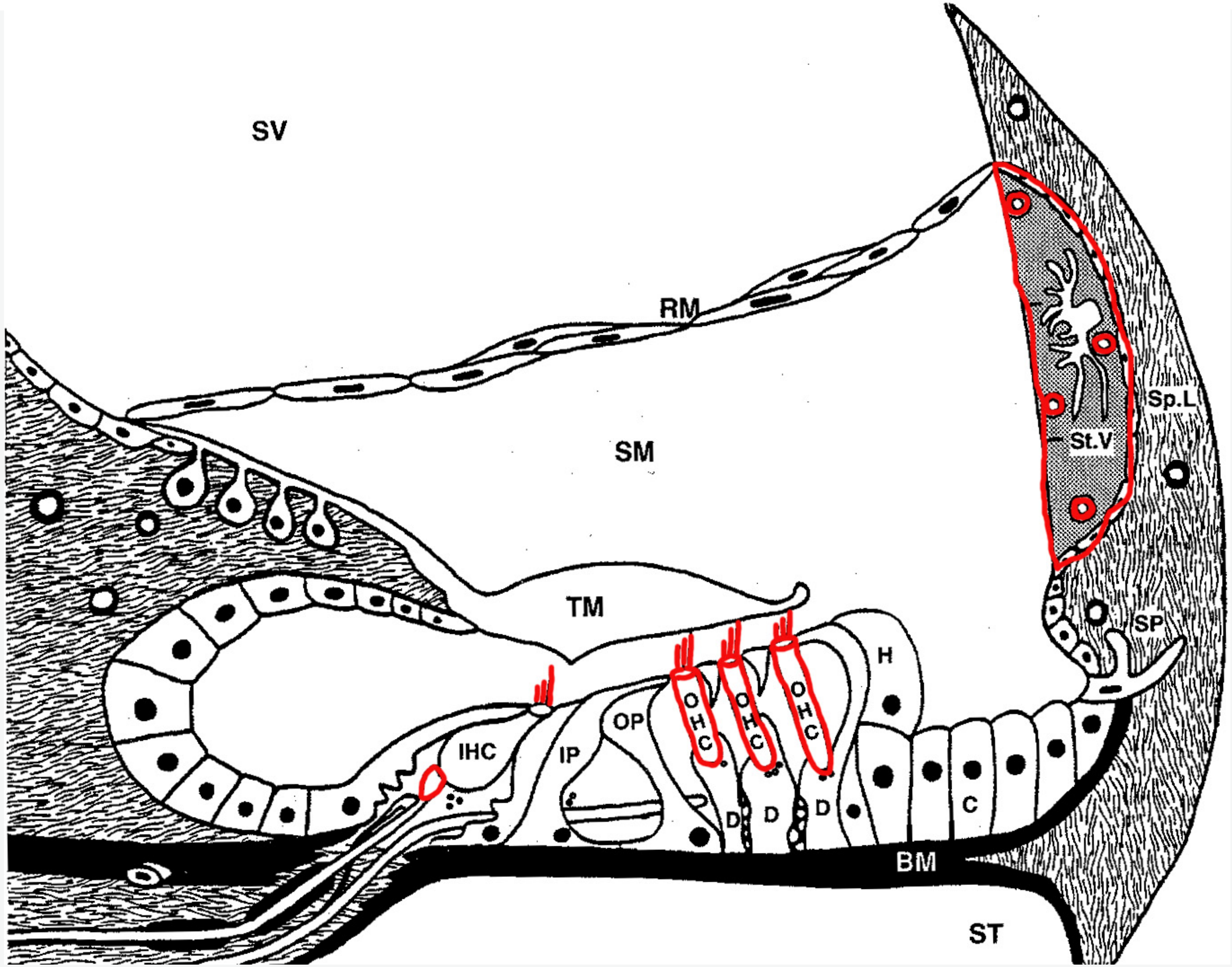
Colleagues in the Office of Academic Affairs

Current and former AuD students

Current and former undergraduate students

Outline

- Introduction and overview of expanding the definition of hearing loss and ototoxicity
- Pre-clinical experiments on the interactions of noise, ototoxic drugs, and aging
- Developing clinically-relevant models mouse models of ototoxicity
- Approaches to preventing ototoxicity
- Identifying new compounds to evaluate for potential ototoxicity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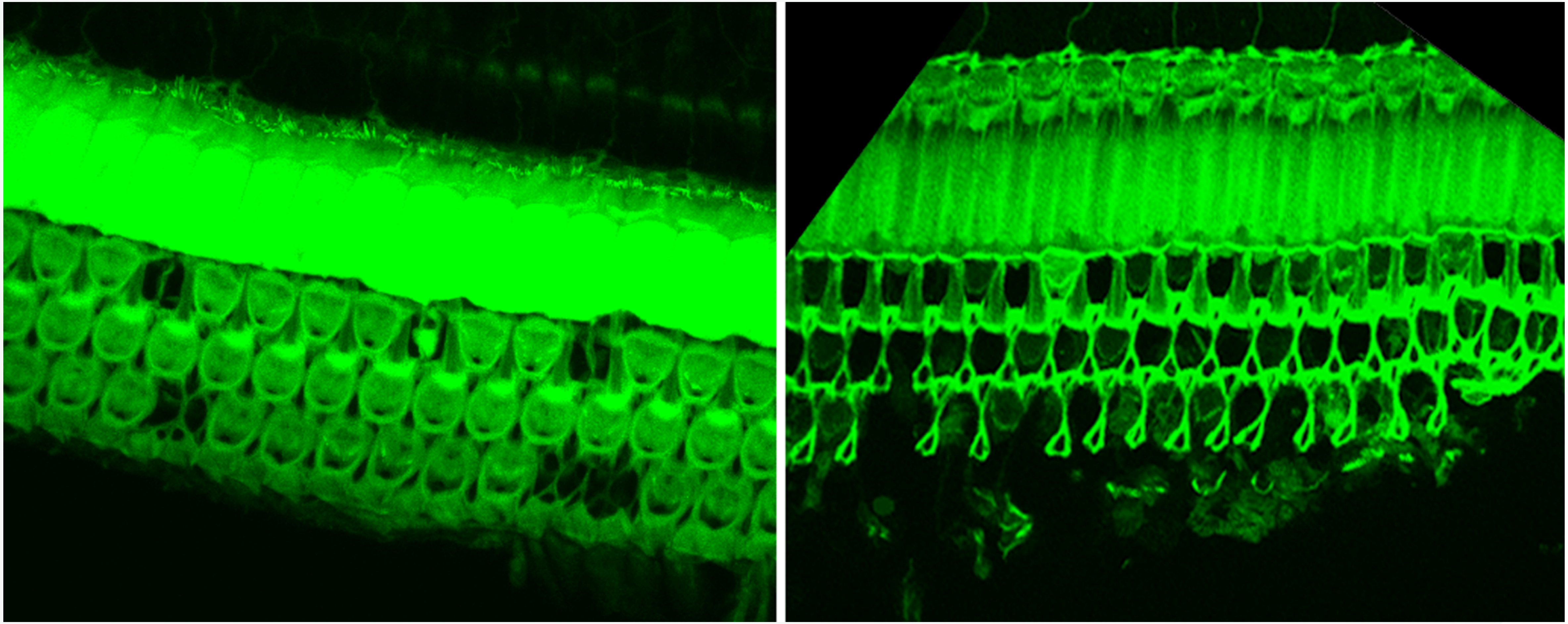
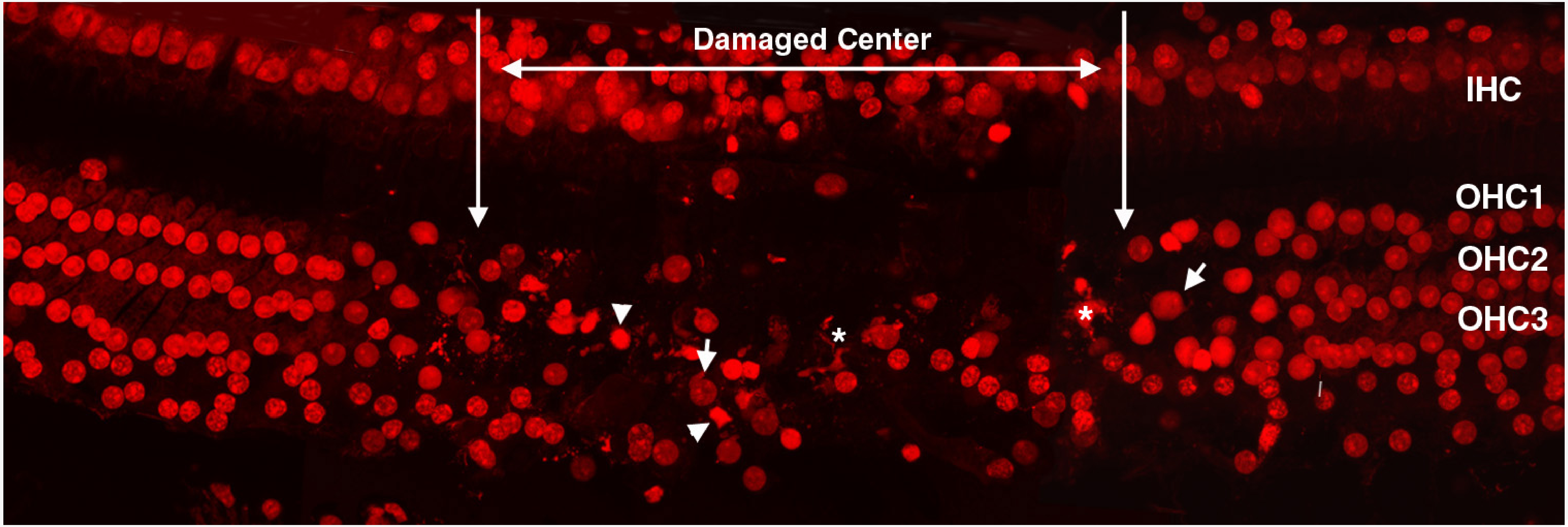
Noise-induced hearing loss



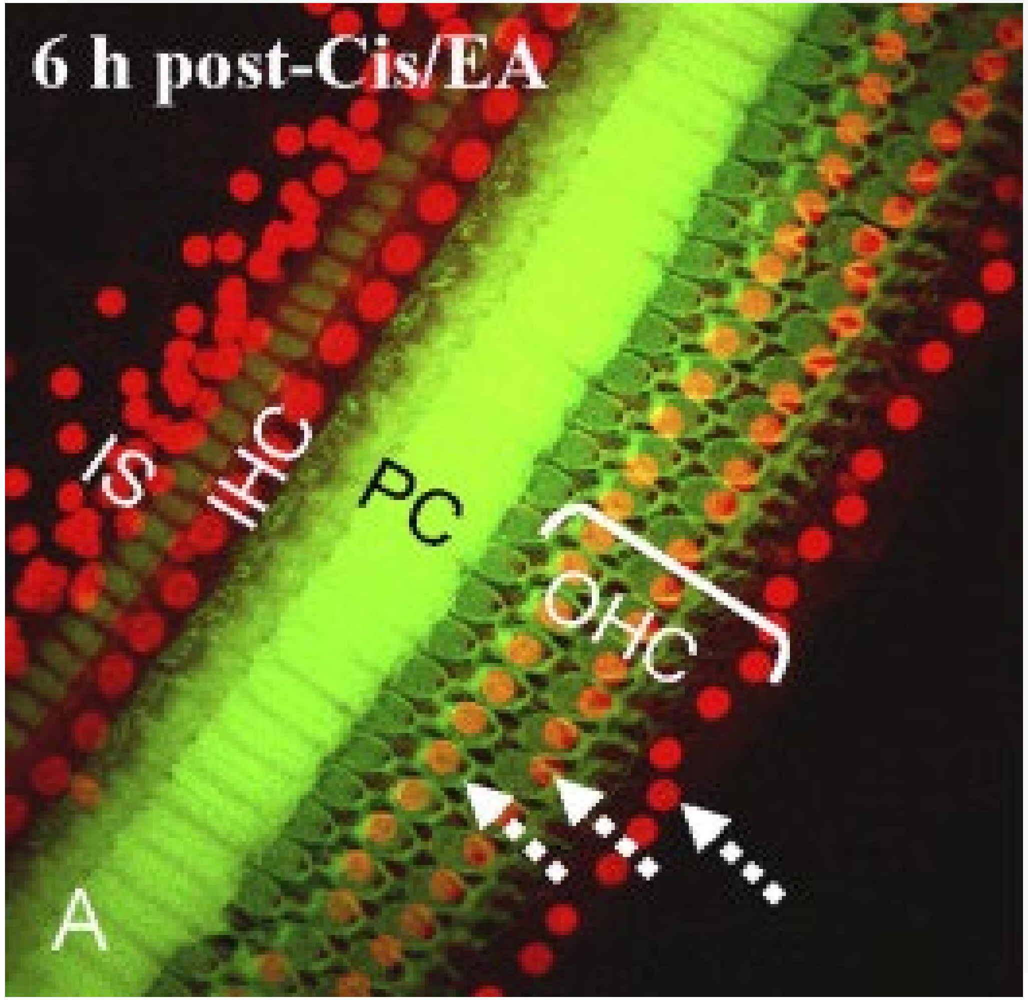
From Henderson, Bielefeld, Harris, Hu (2006) *Ear and Hearing*

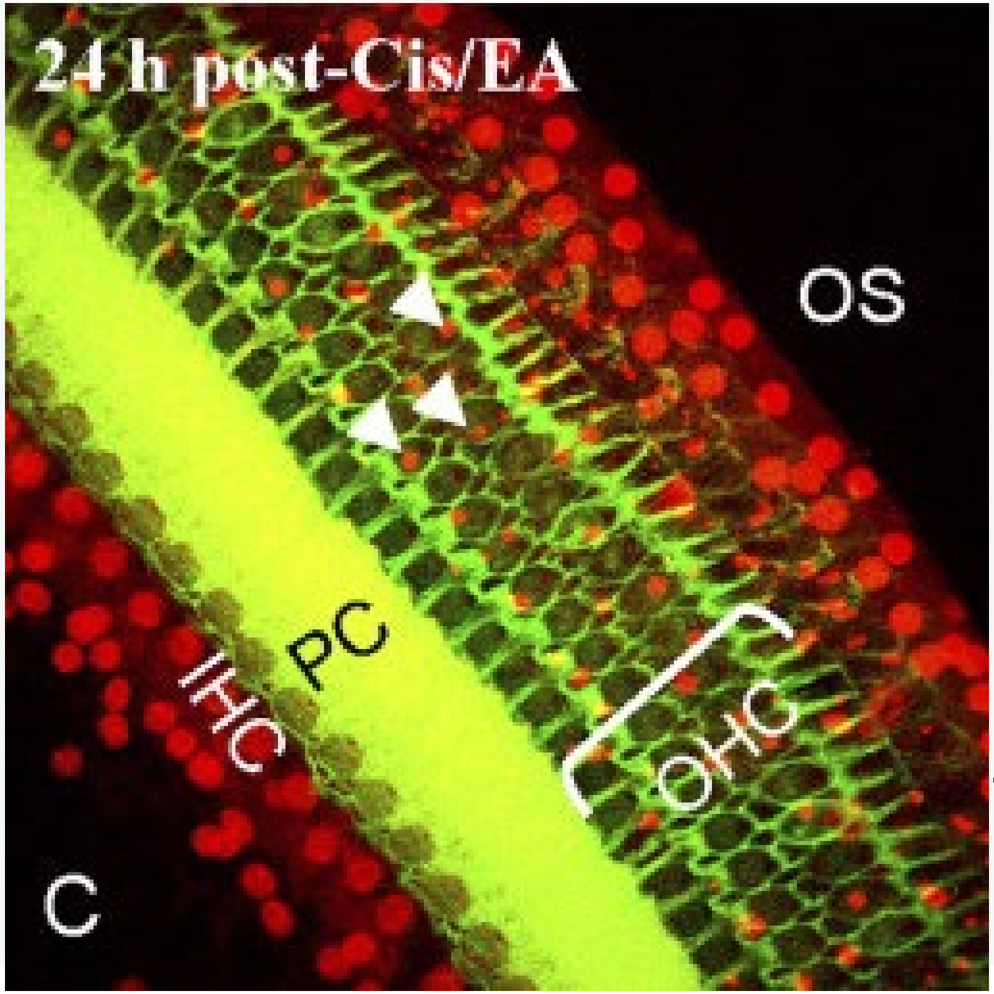
Necrosis and apoptosis in the noise-exposed OHCs



From Henderson, Bielefeld, Harris, Hu (2006) *Ear and Hearing*

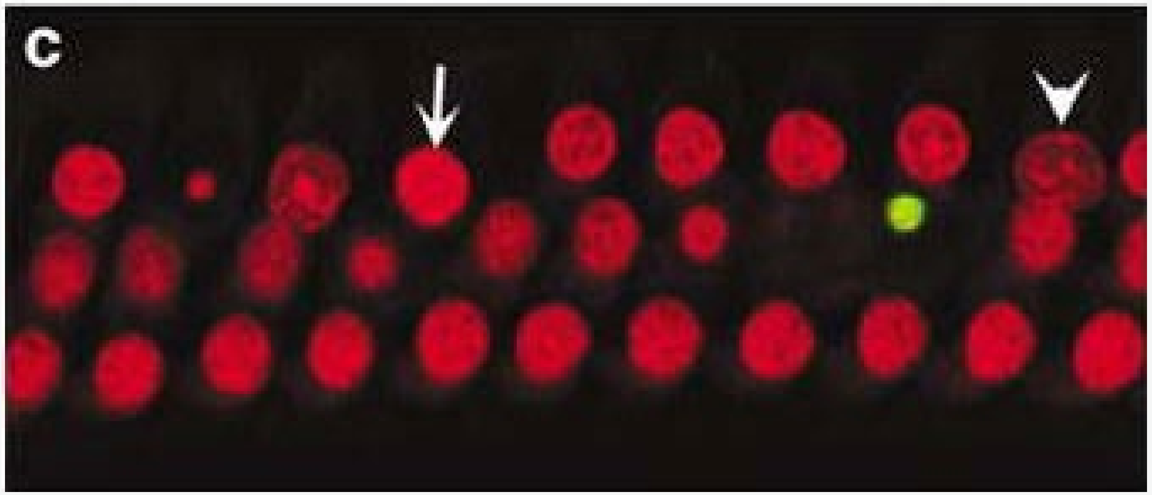
Evidence of Apoptosis in cisplatin-exposed OHC

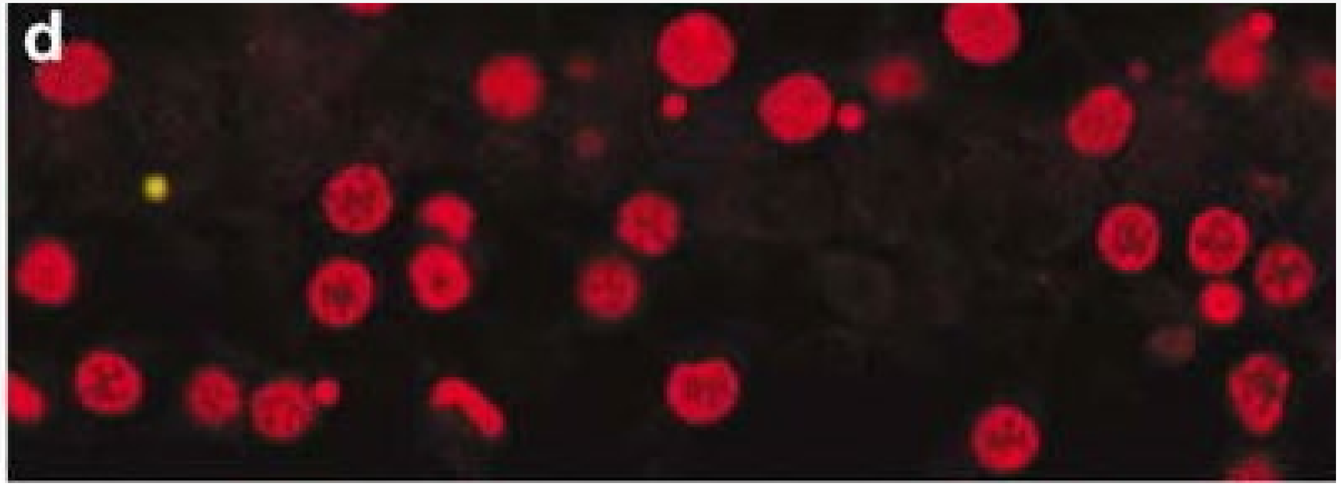




From Ding, Jiang, Wang, and Salvi (2007) Hearing Research 226, 129-139

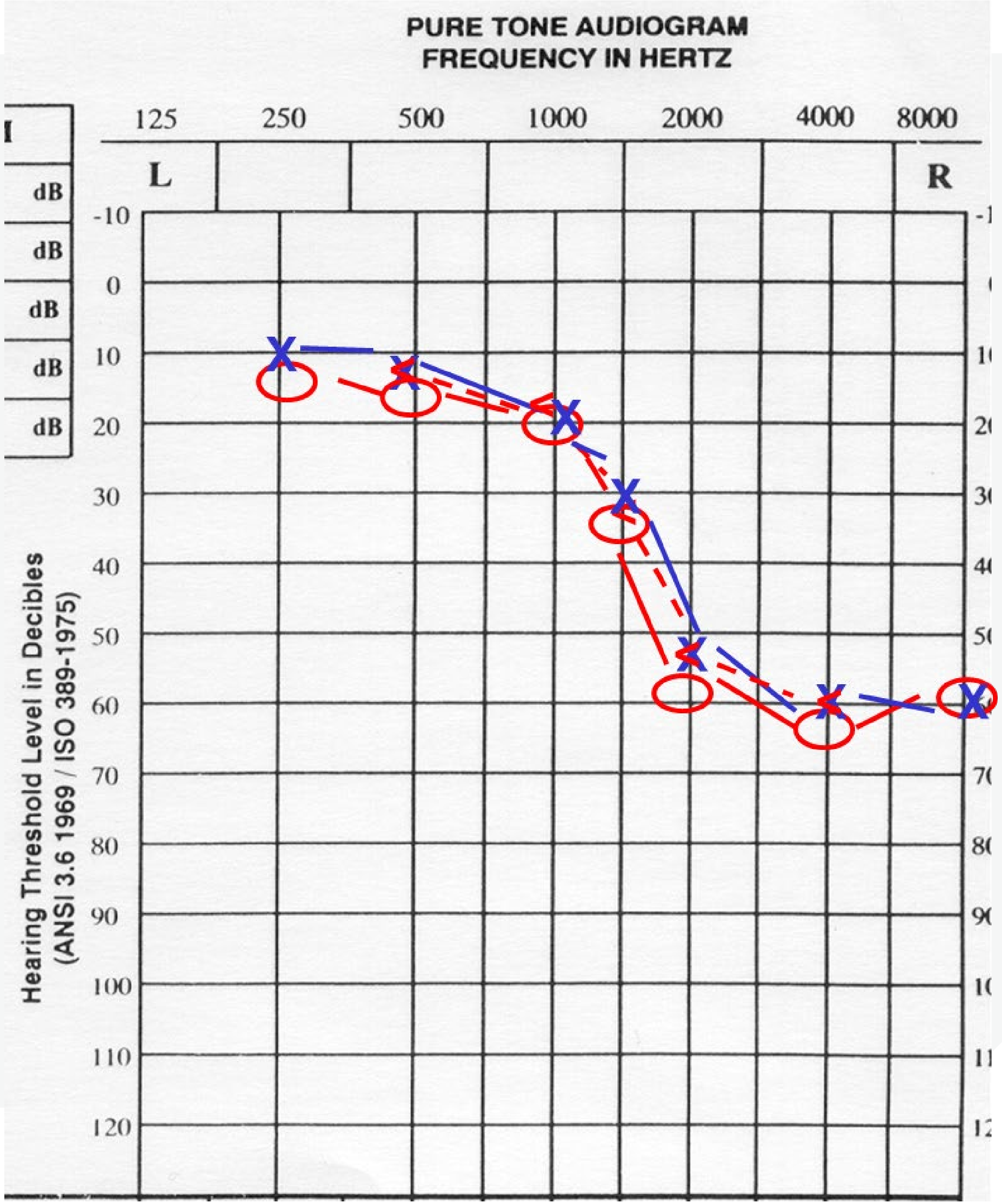
Apoptosis in OHC exposed to kanamycin





From Jiang, Sha, Forge, and Schacht (2006). Cell Death and Differentiation, 13(1), 20-30.

Throughout, the focus was on direct threshold shift from the noise or ototoxic drug



How else should we be thinking about ototoxicity?

- The overall hearing health of our patients over a 9-10 decade lifespan should be part of the mindset
- One additional thought about ototoxicity is how it predisposes the ear to future hearing losses from noise or aging
- Noise-aging interaction can serve as a model

75 year-old woman with no noise exposure/ototoxicity history

Speech Audiometry

Condition	SRT	Discrim	SL	EM
Right	15 dB	88 %	40 dB	dB
Left	10 dB	96 %	40 dB	dB
SF	dB	%	dB	dB
Aided Right	dB	%	dB	dB
Aided Left	dB	%	dB	dB

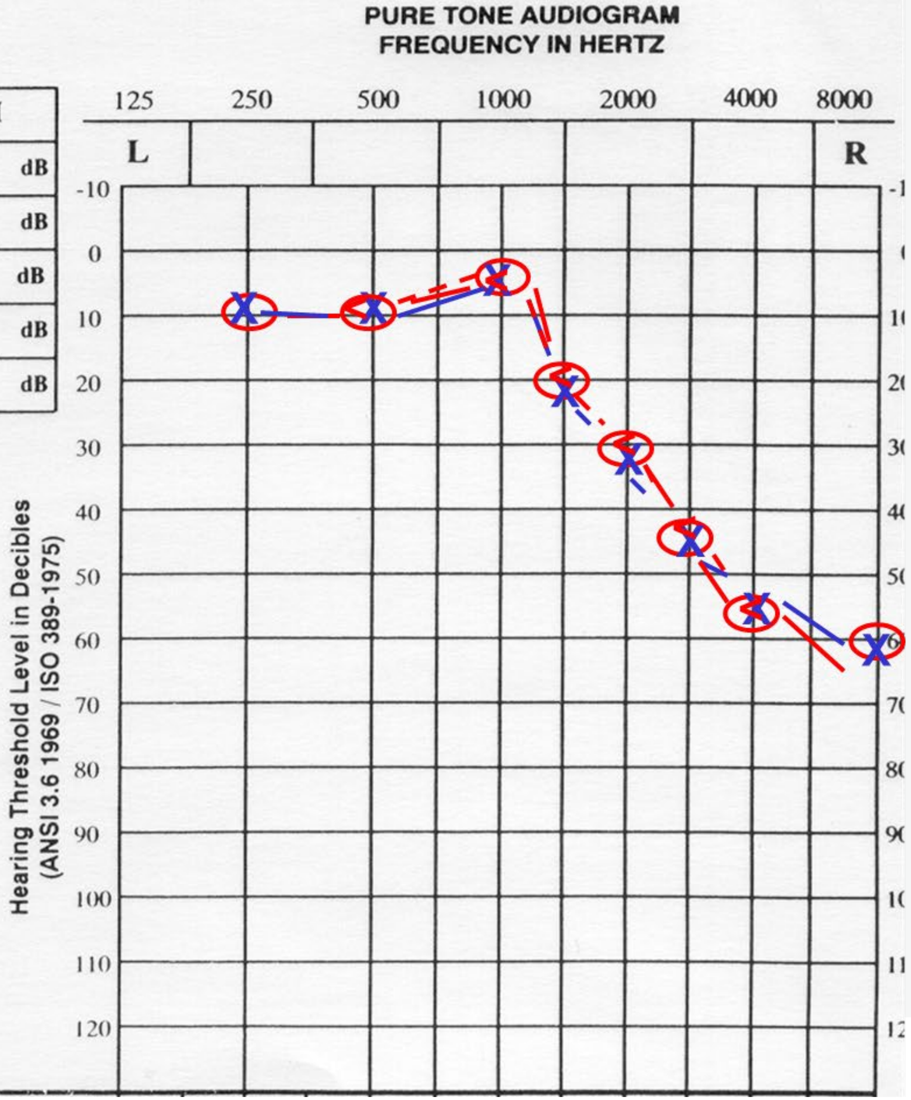
Right Ear MCL _____ dB UCL _____ dBHL
Left Ear MCL _____ dB UCL _____ dB

TYMPANOMETRY

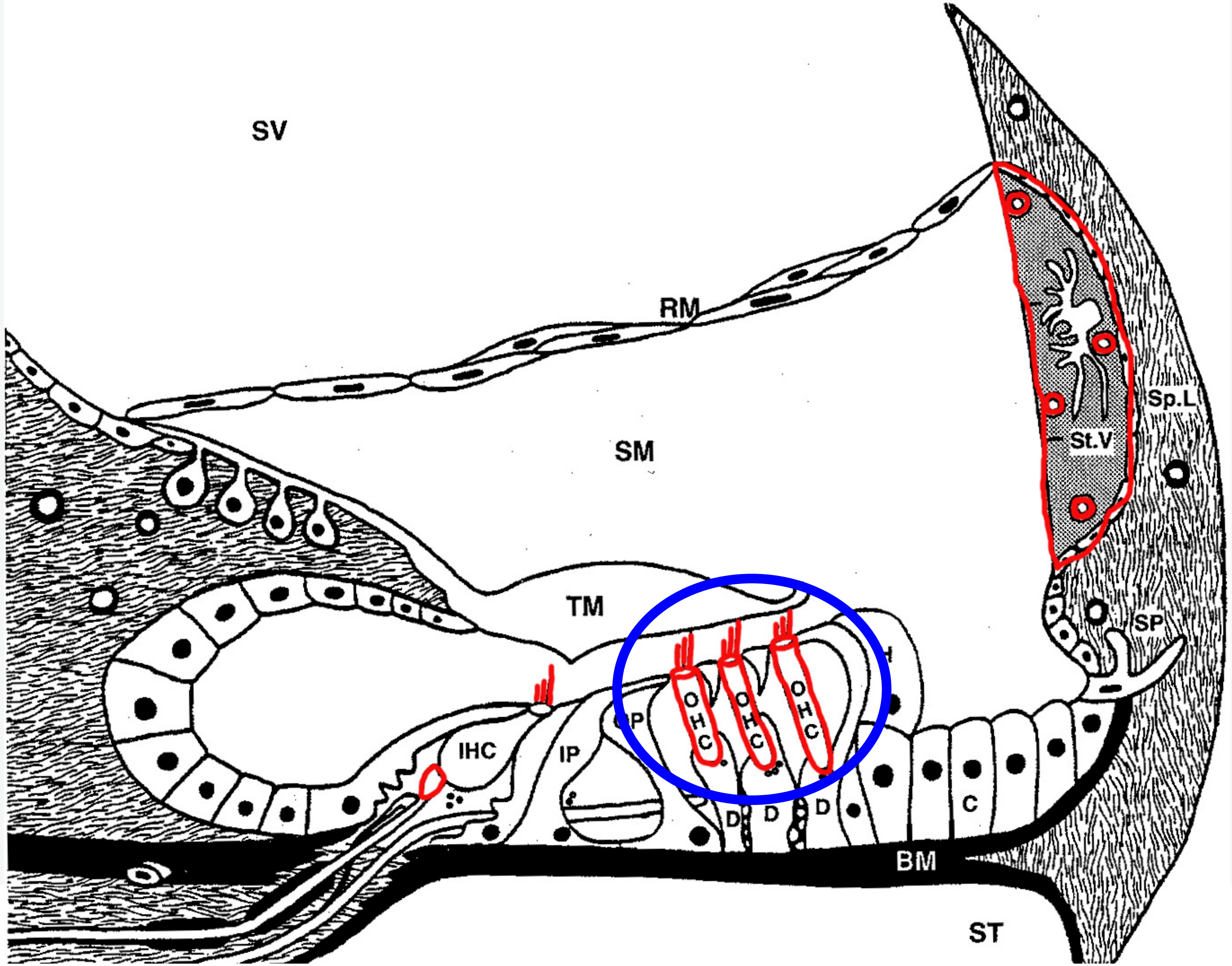
RT Ear Curve A Pressure 5 Vol 1.0
LT Ear Curve A Pressure 5 Vol 1.2

Audiogram Code

EAR	AIR Un-Masked	AIR Masked	BONE Un-Masked	BONE Masked	Sound Field Aided	Sound Field Un Aided
R	O–O	△–△	< - - - <	[[	A	S
L	X – X	□–□	> - - - >	]]	A	S



Outer hair cell pathology- Sensory presbycusis?



85 year-old woman with no noise exposure/ototoxicity history

Speech Audiometry

Condition	SRT	Discrim	SL	EM
Right	40 dB	92 %	40 dB	dB
Left	40 dB	92 %	40 dB	dB
SF	dB	%	dB	dB
Aided Right	dB	%	dB	dB
Aided Left	dB	%	dB	dB

Right Ear MCL _____ dB UCL _____ dBHL

Left Ear MCL _____ dB UCL _____ dB

TYMPANOMETRY

RT Ear Curve A Pressure -60 Vol 1.3

LT Ear Curve A Pressure -45 Vol 1.1

Audiogram Code

EAR	AIR Un-Masked	AIR Masked	BONE Un-Masked	BONE Masked	Sound Field Aided	Sound Field Un Aided
R	O–O	△–△	< - - - <	[[	A	S
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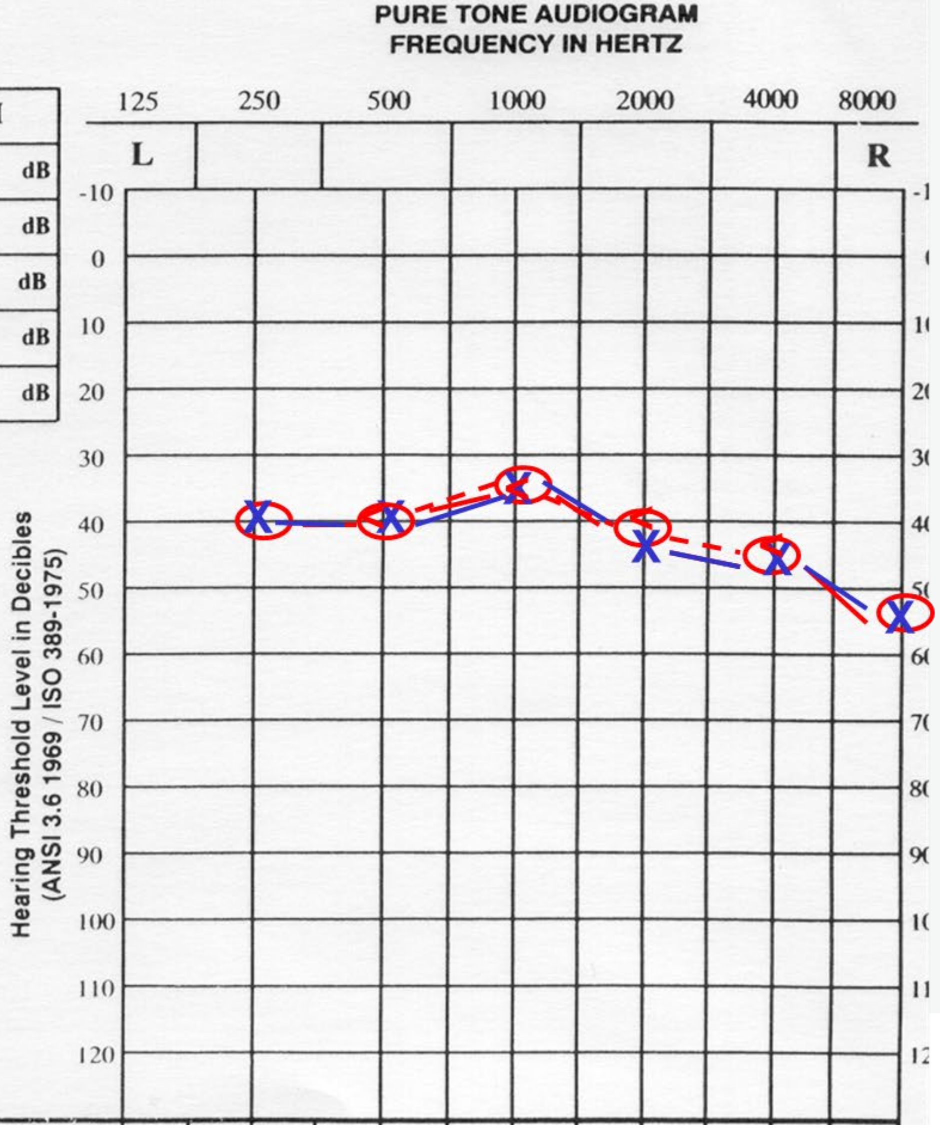
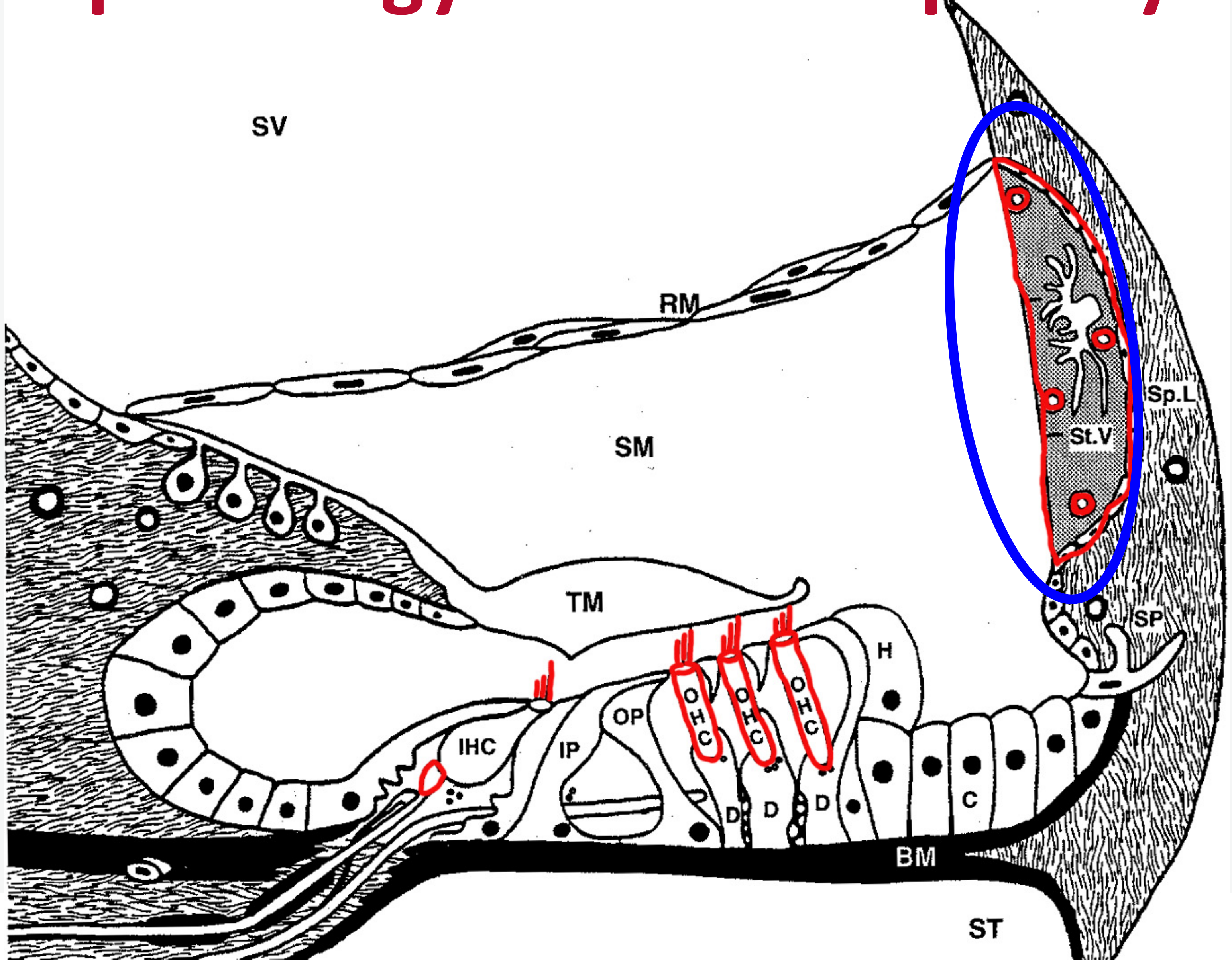


Lateral wall pathology- Metabolic presbycusis?



86 year-old man with possible early-age noise exposure history

Speech Audiometry

Condition	SRT	Discrim	SL	EM
Right	65 dB	28 %	20 dB	dB
Left	70 dB	36 %	20 dB	dB
SF	dB	%	dB	dB
Aided Right	dB	%	dB	dB
Aided Left	dB	%	dB	dB

Right Ear MCL _____dB UCL _____dBHL

Left Ear MCL ______ dB UCL _____ dB

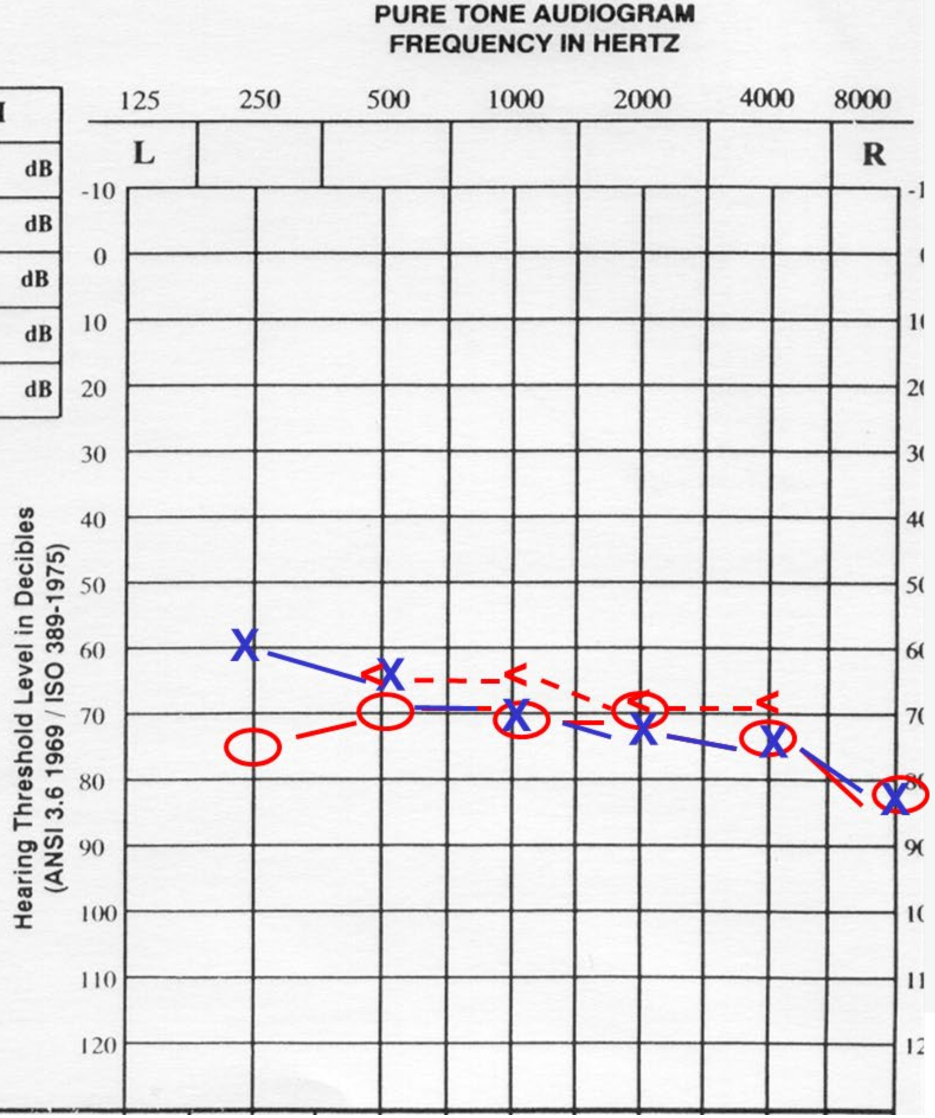
TYMPANOMETRY

RT Ear Curve A Pressure 60 Vol 1.4

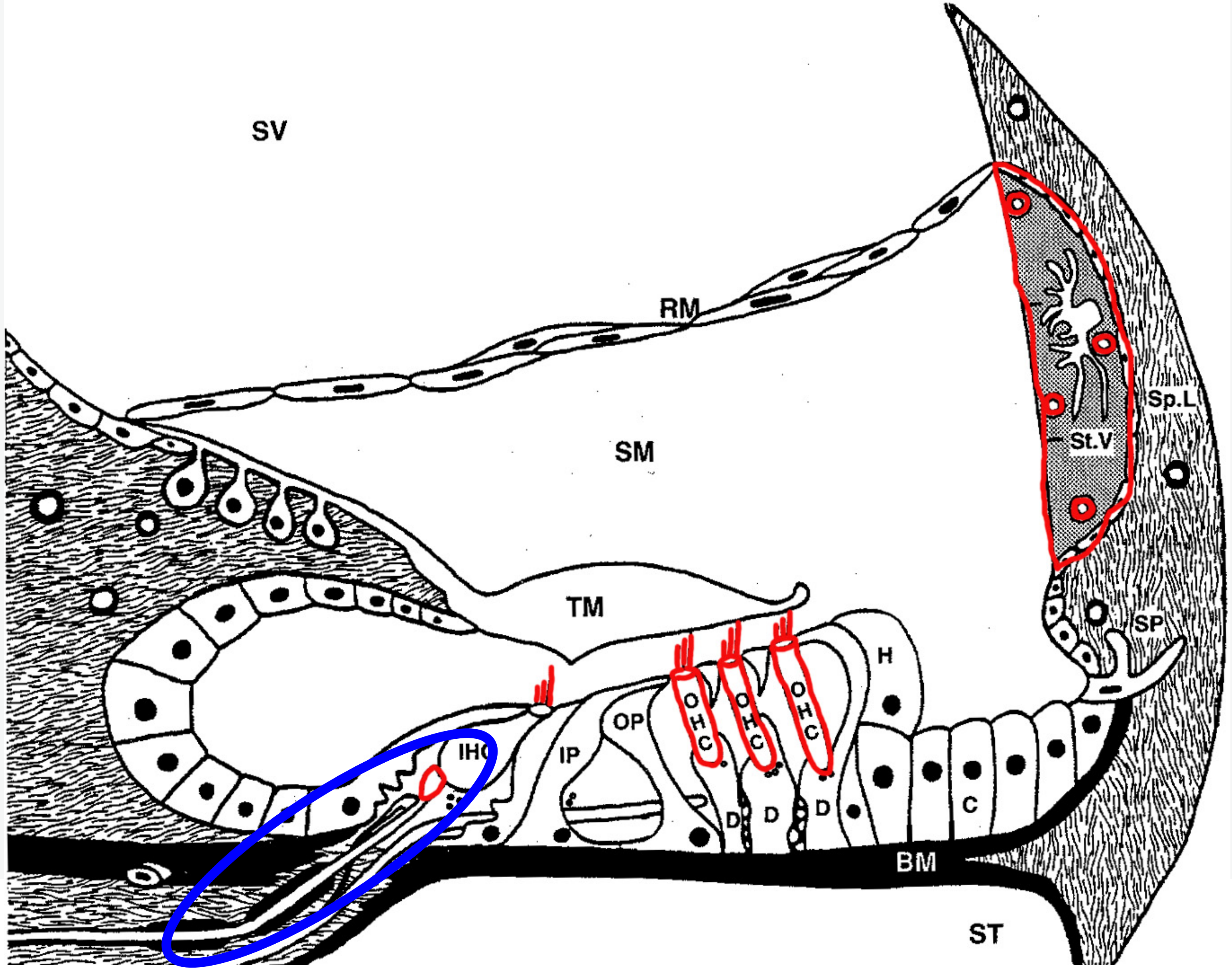
LT Ear Curve A Pressure 45 Vol 1.3

Audiogram Code

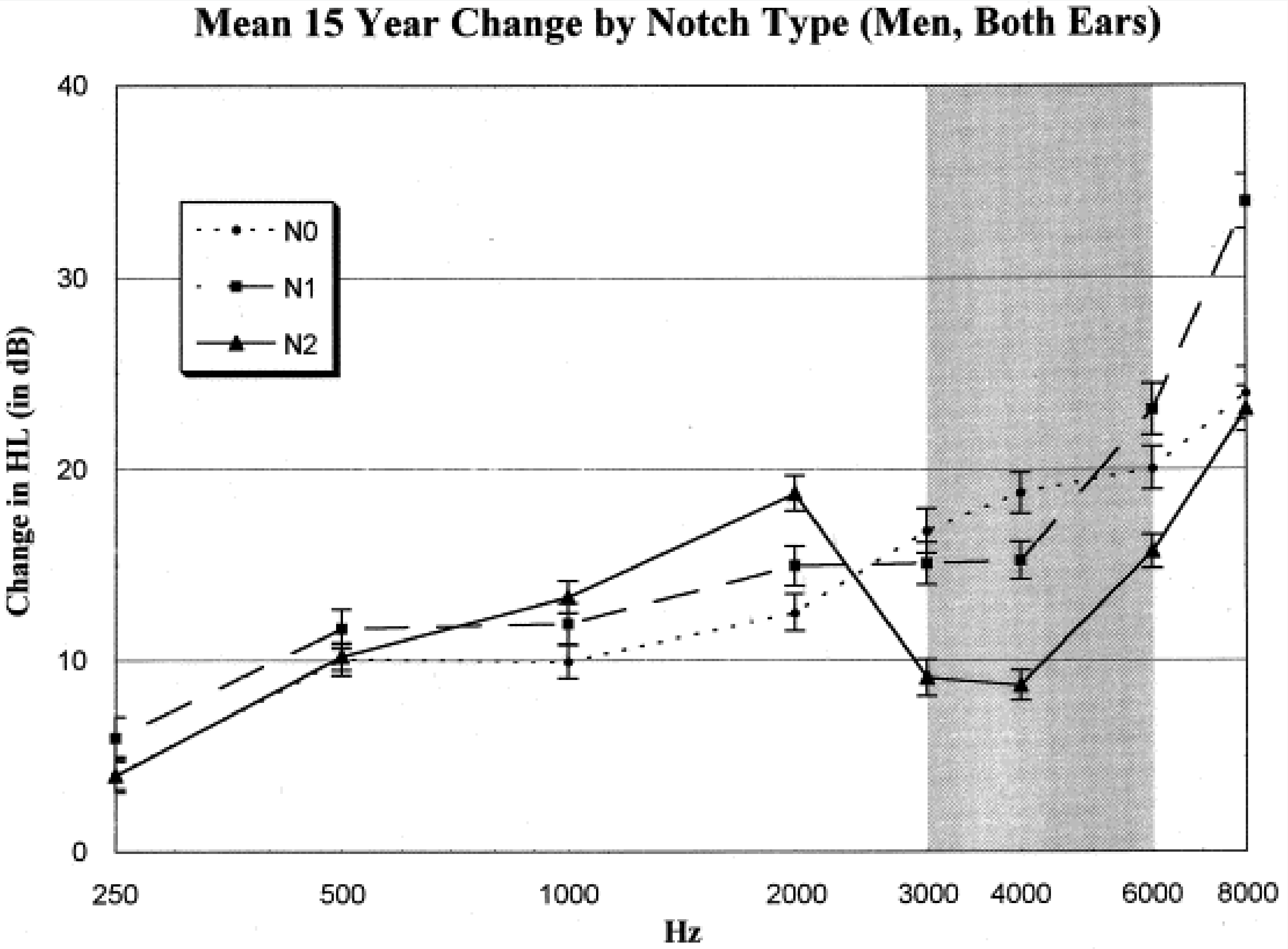
EAR	AIR Un-Masked	AIR Masked	BONE Un-Masked	BONE Masked	Sound Field Aided	Sound Field Un Aided
R	O–O	△–△	< - - - <	[[	A	S
L	x – x	□–□	> - - - >	]]	A	S



Afferent auditory nerve pathology - Neural presbycusis?



Interaction of noise and aging on thresholds is complex



From Gates, G.A., Cooper, J.C. Jr, Kannel, W.B., & Miller, N.J. (1990). *Ear and Hearing,* 11, 247-256.

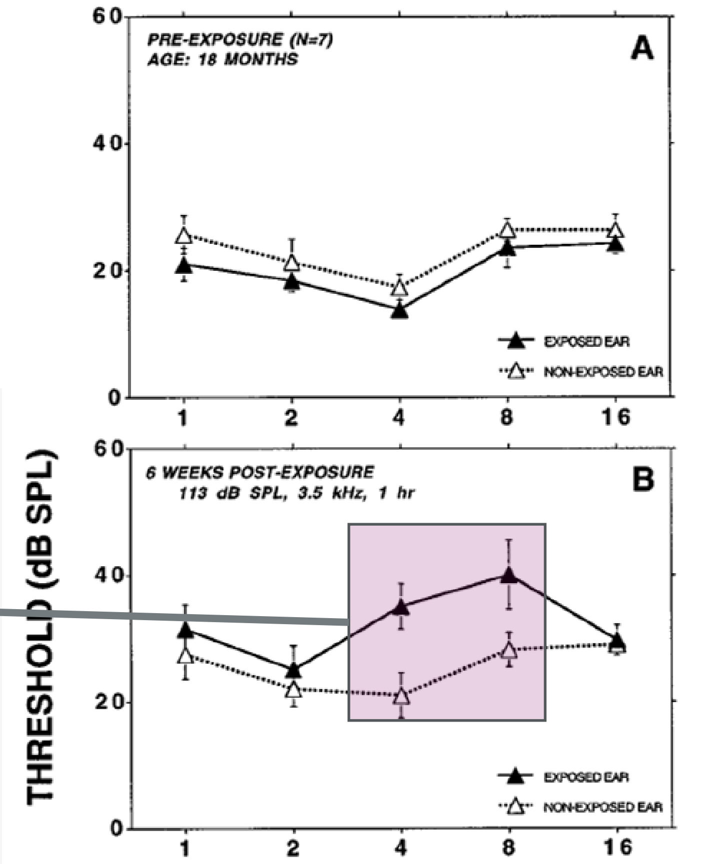
Human data

- Noise-induced hearing loss (NIHL) reduces age-related hearing loss (ARHL) in the noise notch

- NIHL increases ARHL in the adjacent frequency

Impact of NIHL on ARHL may depend on pathology of ARHL

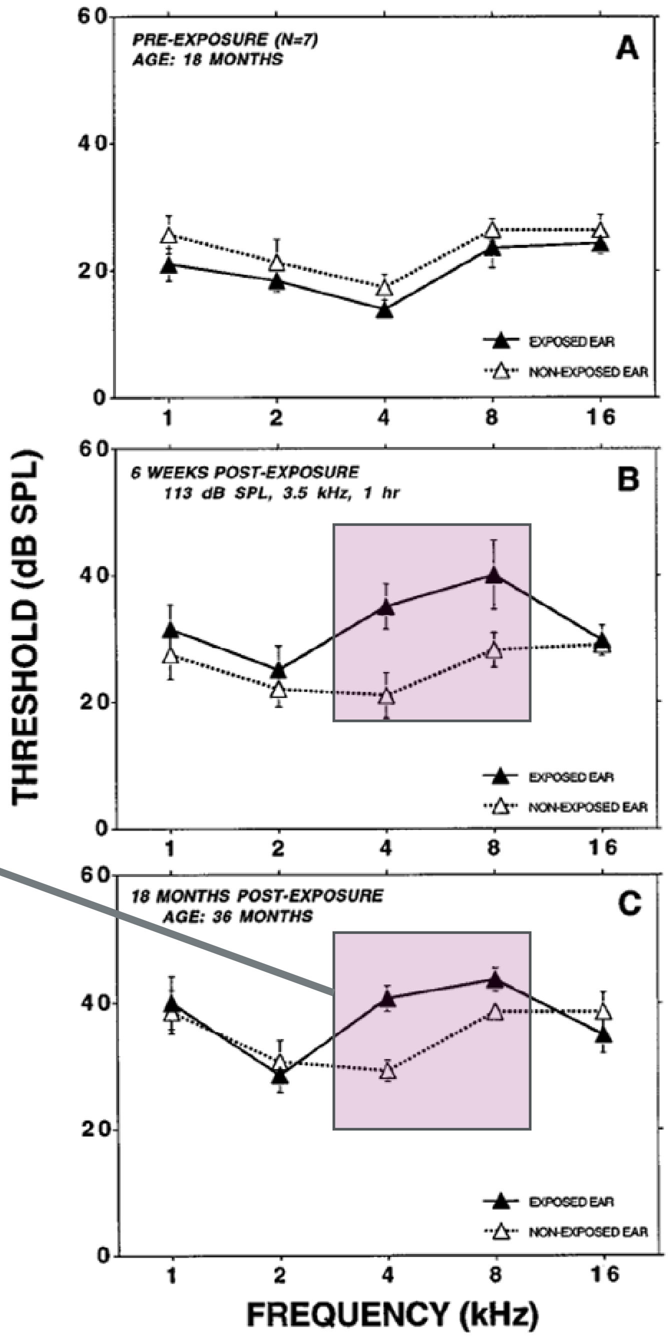
Noise-exposed Mongolian gerbil ears show significantly higher thresholds at 6 weeks after noise



From Mills, J.H., Boettcher, F.A., Dubno, J.R. 1997. *JASA*, 101, 1681-6.

Impact of NIHL on ARHL may depend on pathology of ARHL

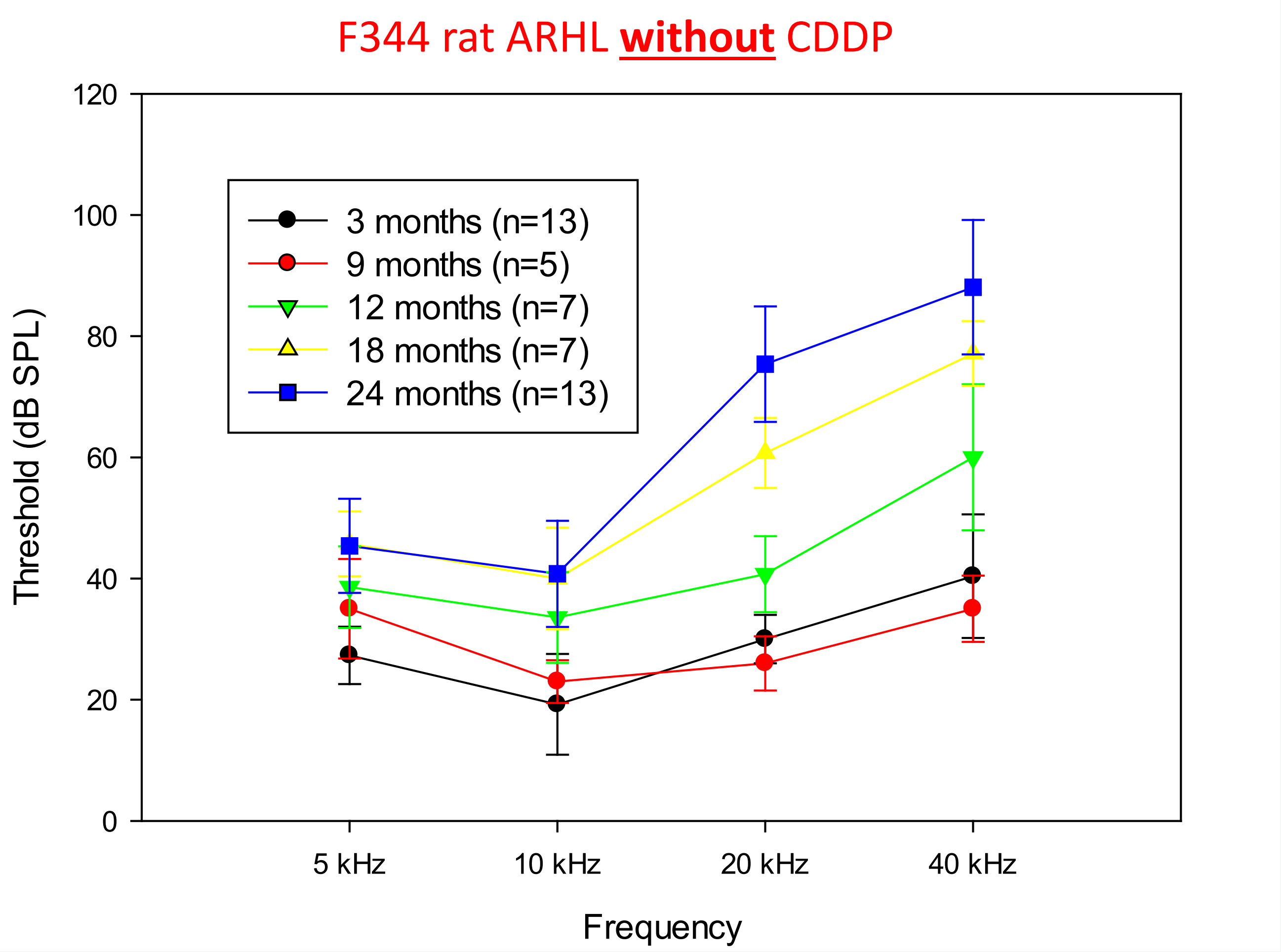
Non-noise-exposed ears show significantly higher threshold shift from 6 weeks to 18 months post exposure



From Mills et al. (1997)

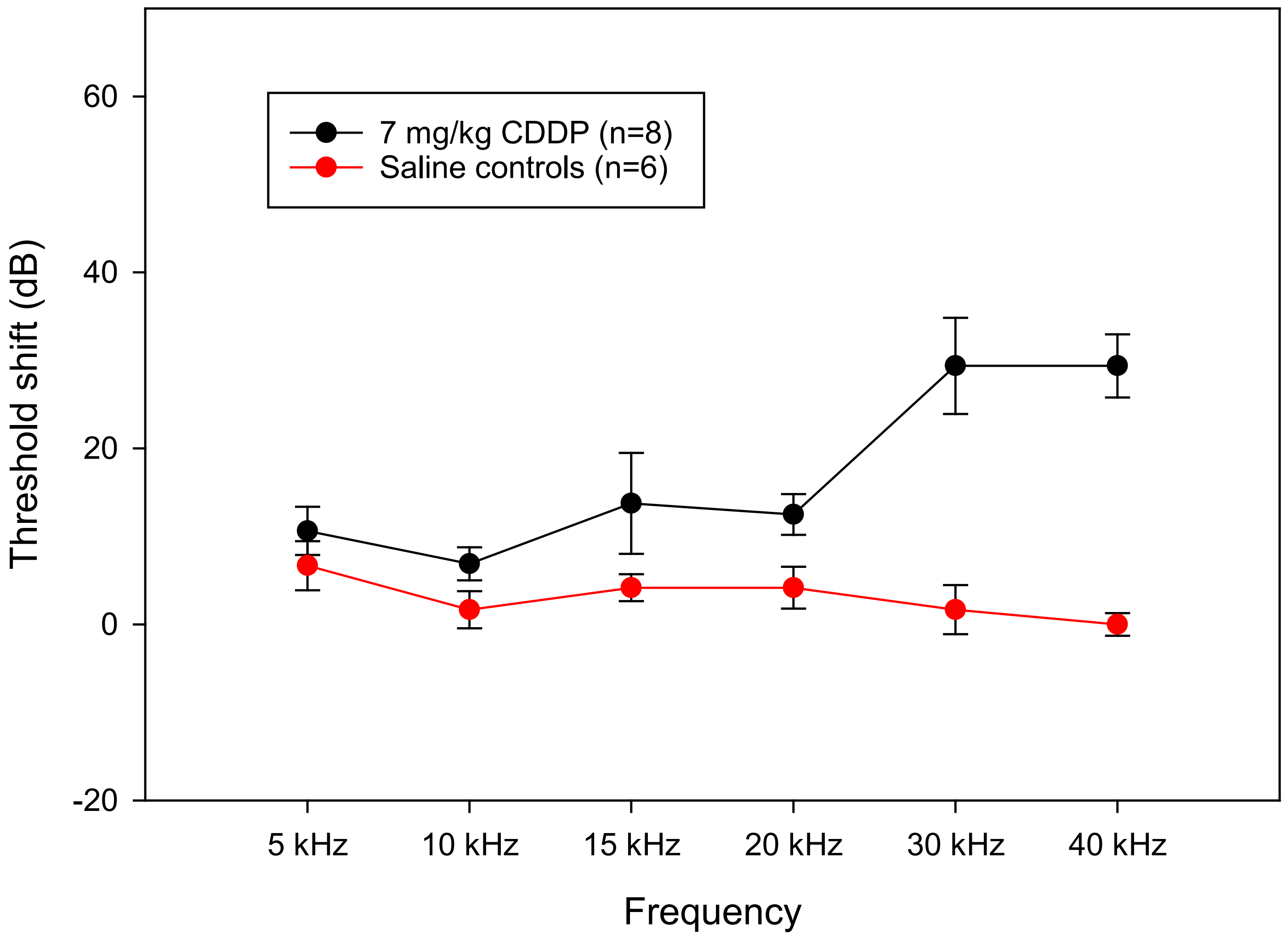
Cisplatin ototoxicity – aging interaction

- Fischer 344 rats dosed with 7 mg/kg CDDP via i.p. injection at age 7 months
- Tracked through age 18 months

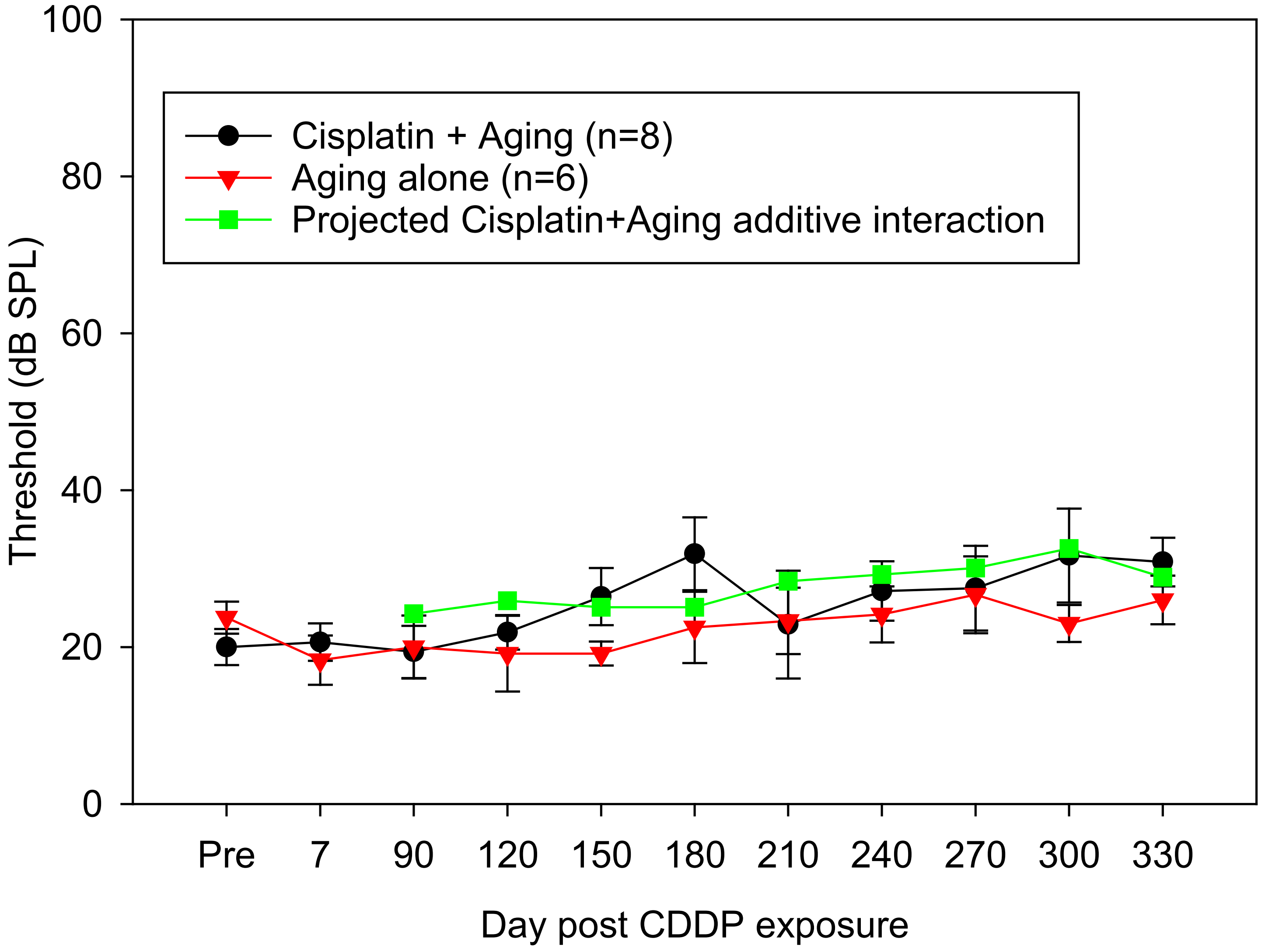


From Bielefeld, Coling, Chen, Li, Tanaka, Hu, Henderson (2008). *Hearing Research*, 245, 48-57.

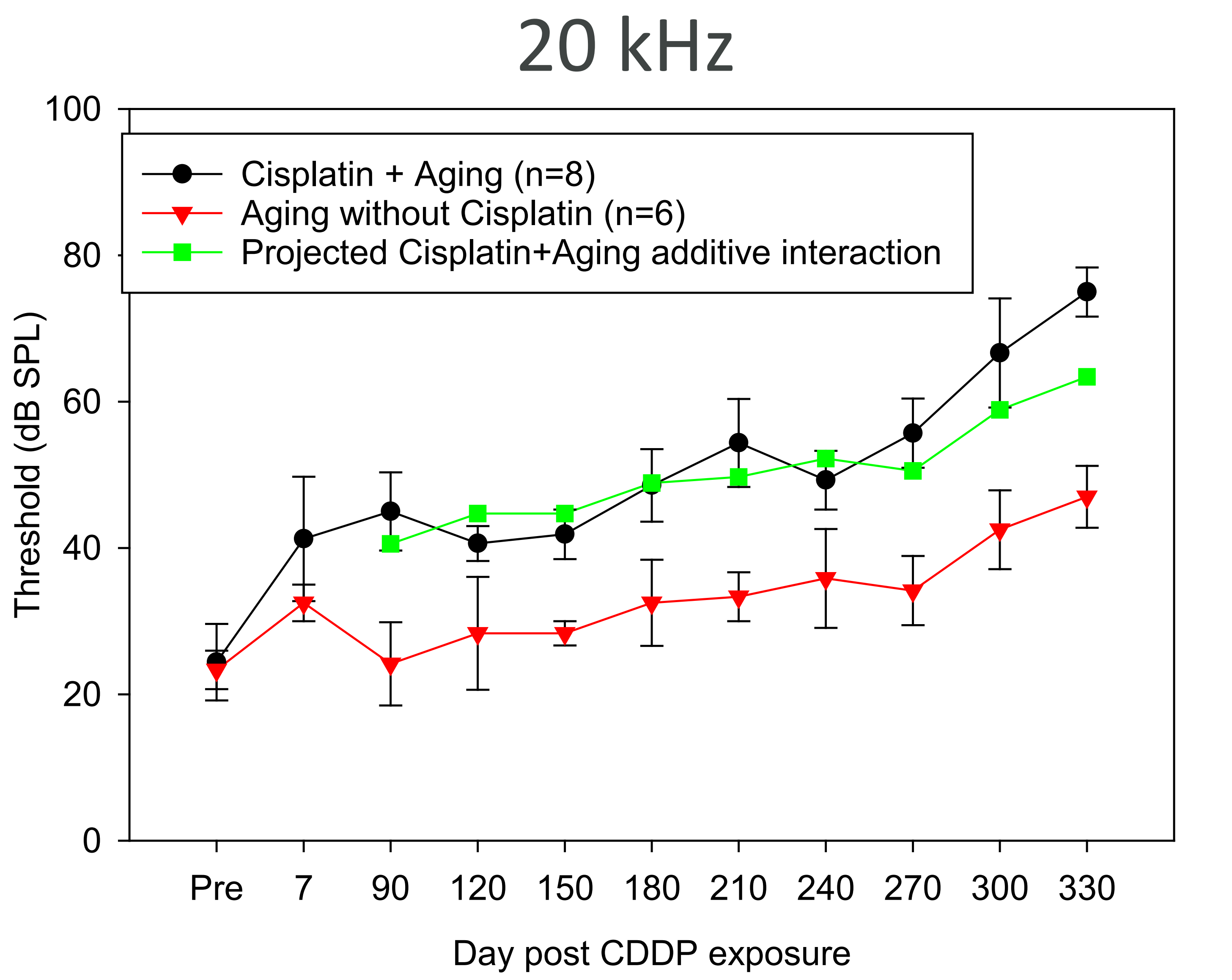
Acute threshold shift from cisplatin



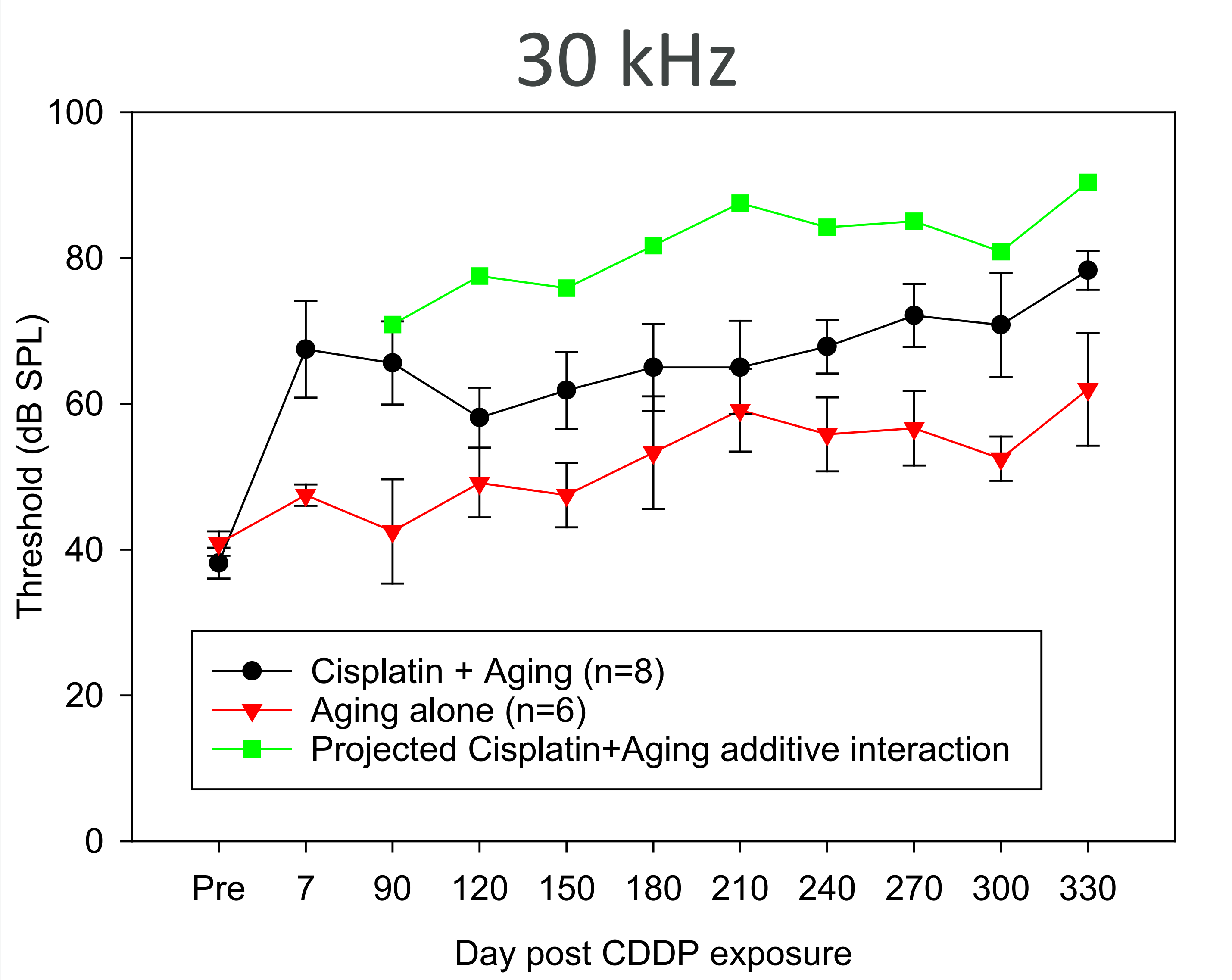
10 kHz



From Bielefeld (2013). *Hearing Research*, 306, 46-53.



From Bielefeld (2013)



From Bielefeld (2013)

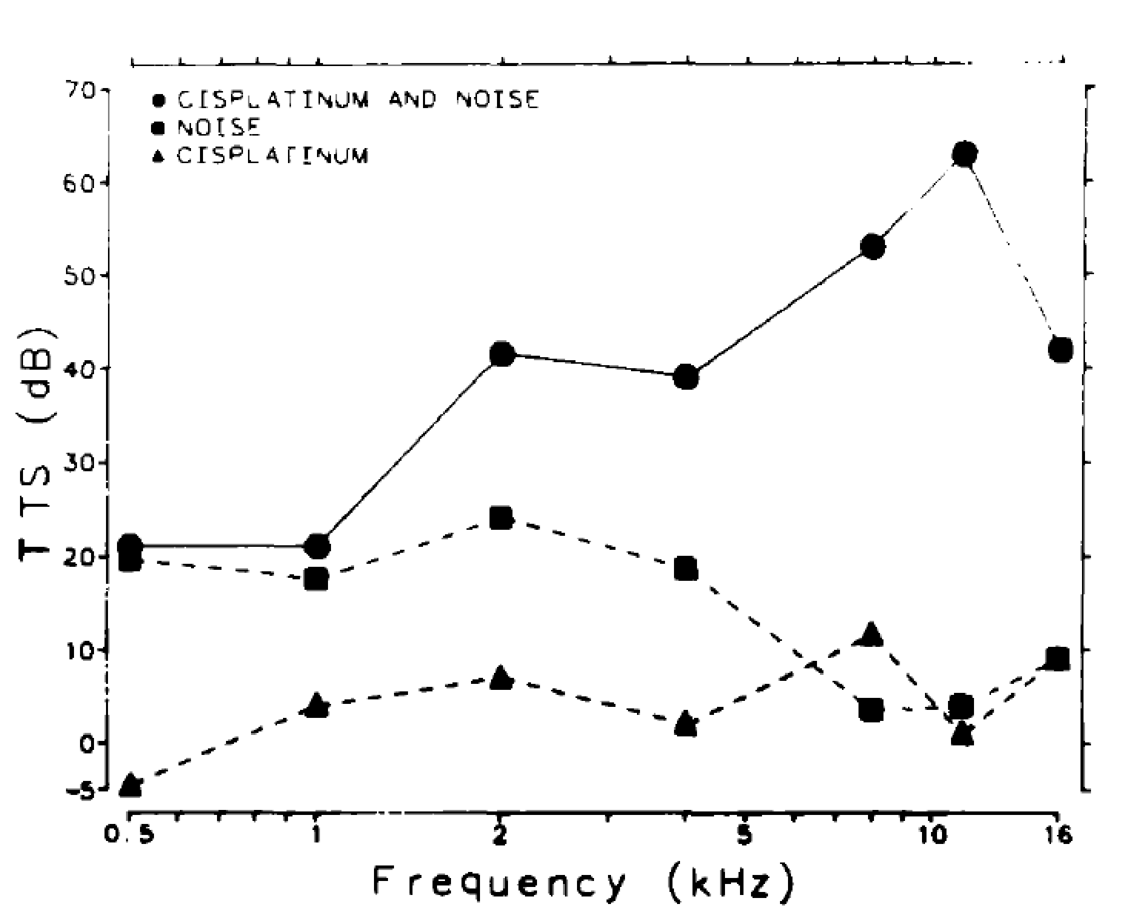
Rat Cisplatin-aging interaction

- Less threshold shift in the affected high frequencies
- More threshold shift at 20 kHz – the transition point of the acute threshold shift
- Similar to the Gates et al. 1990 data in humans with noise history

Cisplatin ototoxicity – noise interaction

- Concurrent noise-CDDP synergistic interaction is well-documented

- If CDDP injury can accelerate ARHL, could CDDP permanently pre-dispose ear to NIHL?



From Boettcher, Henderson, Gratton, Danielson, Byrne (1987). *Ear and Hearing*, 8, 192-212.

Experiment:

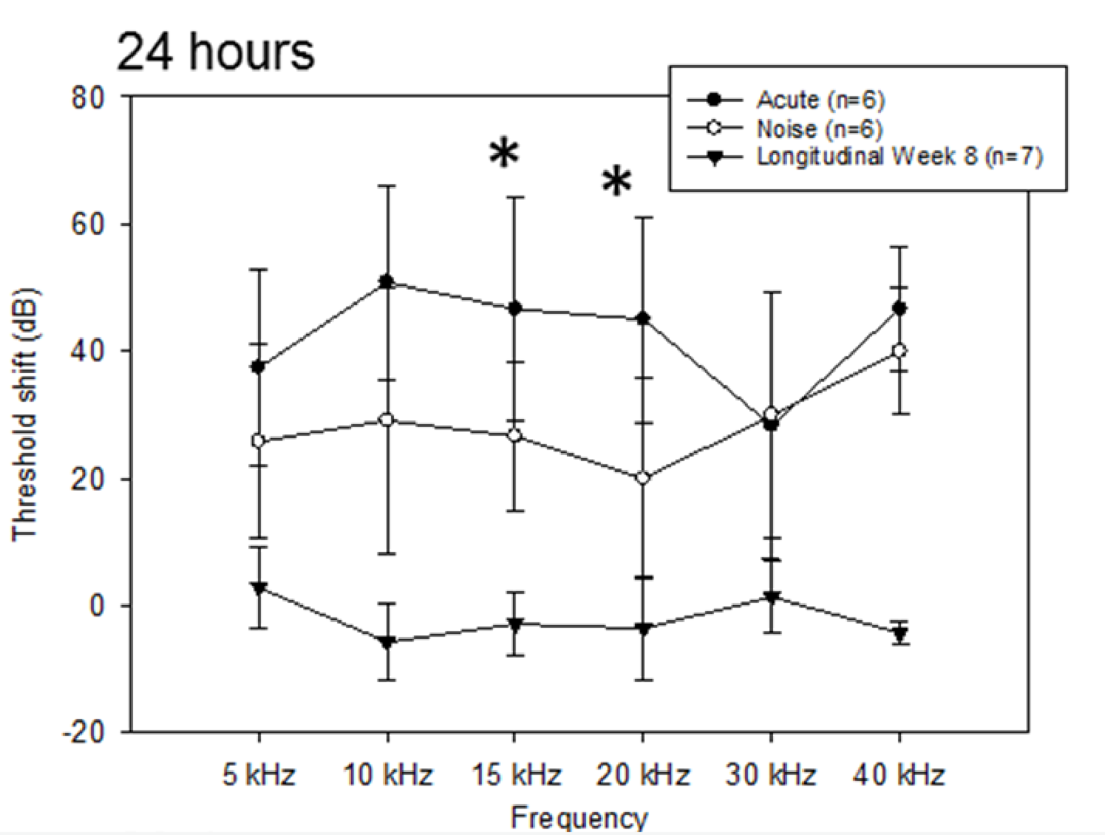
- Fischer 344/NHsd rats
- Cisplatin: 10 mg/kg cumulative dose over 8 weeks
- Noise: 10 kHz, 2-OBN, at 110 dB SPL for 2 hrs

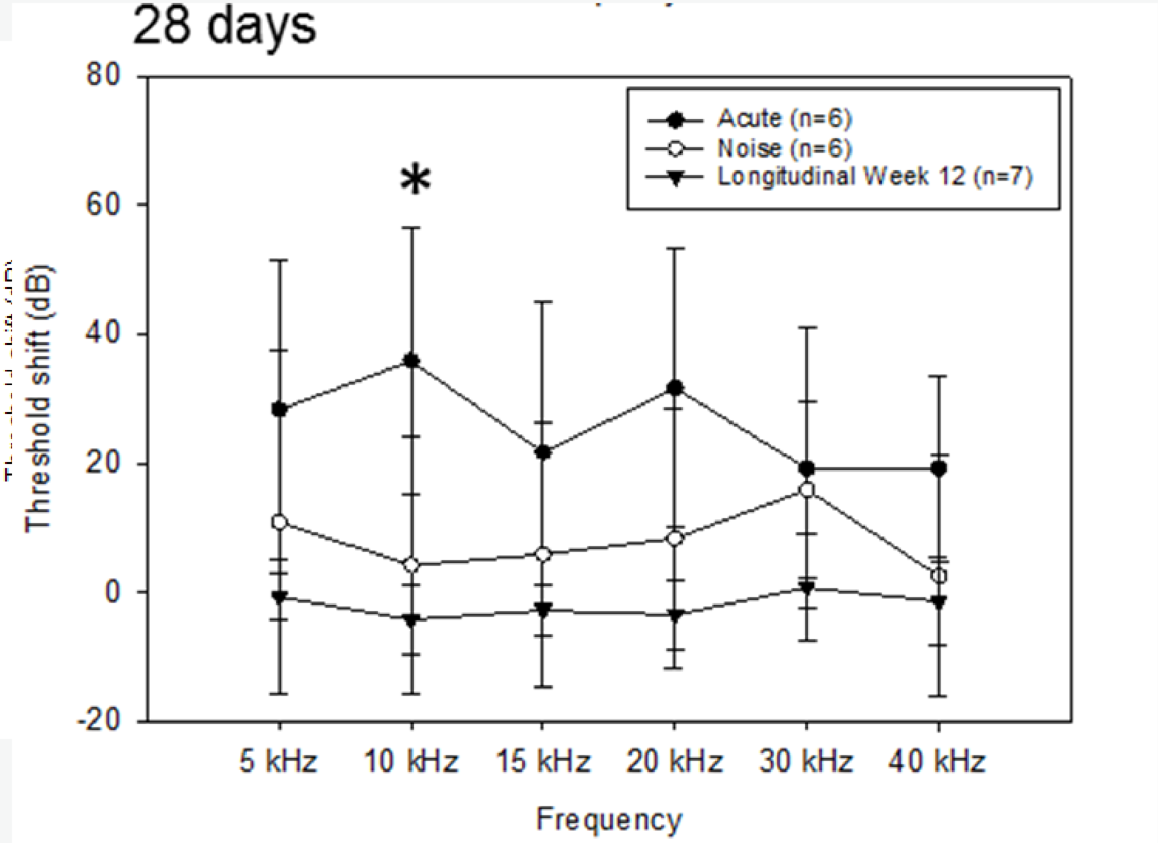
Five groups:

From DeBacker, Harrison, Bielefeld (2017). *Ear and Hearing*, 38, 282-291

Group	Exposure and Testing Paradigm			
CDDP	Cisplatin	ABR and Tissue Collection		
Noise	x	Noise	ABR and Tissue Collection	
Acute	Cisplatin	Noise	ABR and Tissue Collection	
Delayed	Cisplatin	16-week Monitoring Period	Noise	ABR and Tissue Collection
Longitudinal	Cisplatin	20-week Monitoring Period		ABR and Tissue Collection

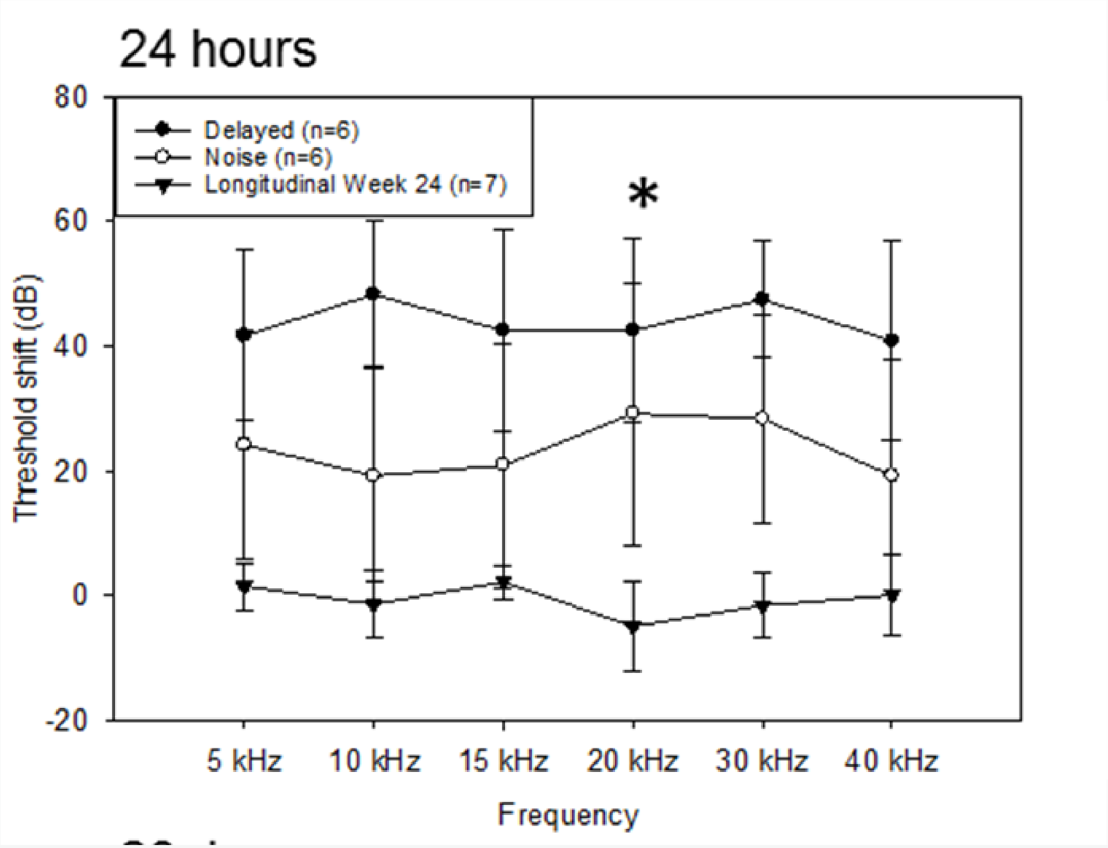
Acute group noise-induced threshold shifts

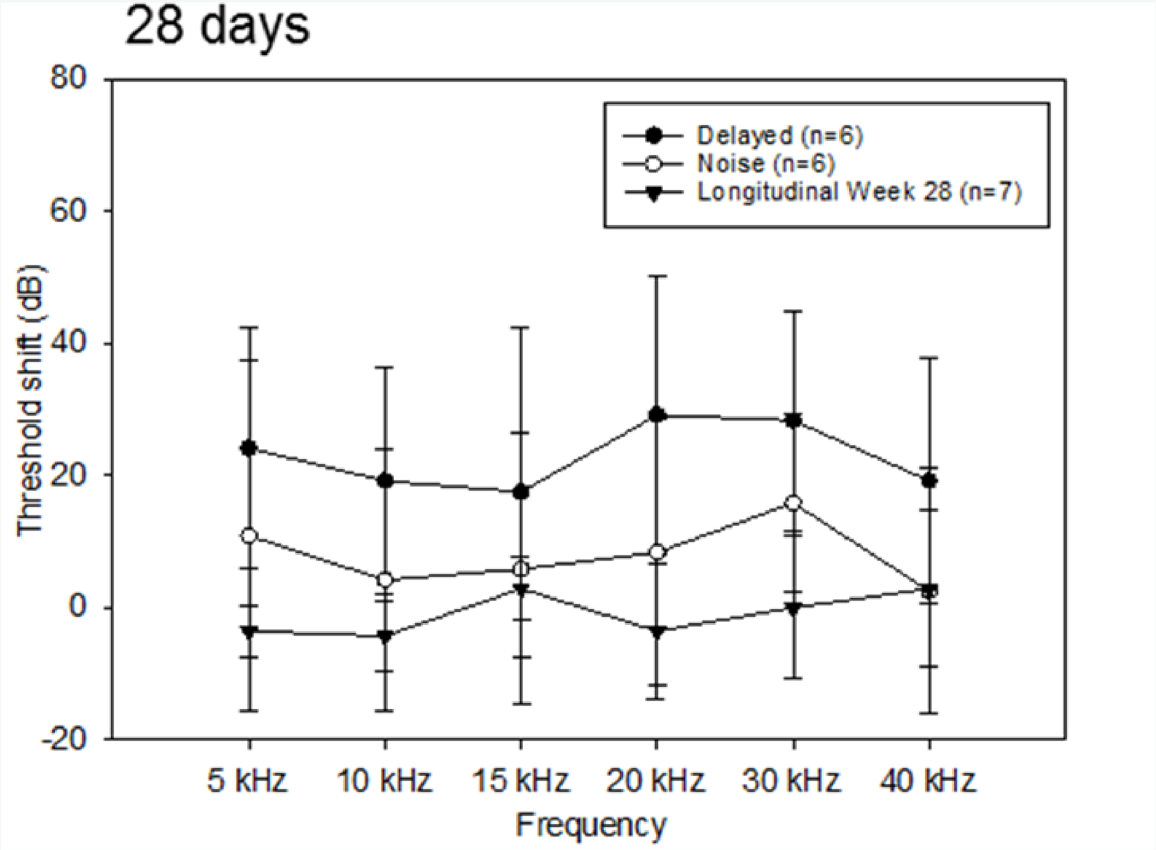




From DeBacker et al. (2017)

Delayed group noise-induced threshold shifts





From DeBacker et al. (2017)

As we think about cisplatin interactions, we need clinically-relevant animal models of cisplatin exposure

- Which mouse model most efficiently and effectively models human cisplatin-induced hearing loss?
- What mode of cisplatin delivery creates an appropriate hearing loss with minimal mortality?

Accumulated cisplatin ototoxicity in mouse models

3 mouse strains – all from Jackson Labs

Given 3 cycles of 16 mg/kg cisplatin by i.p. injection

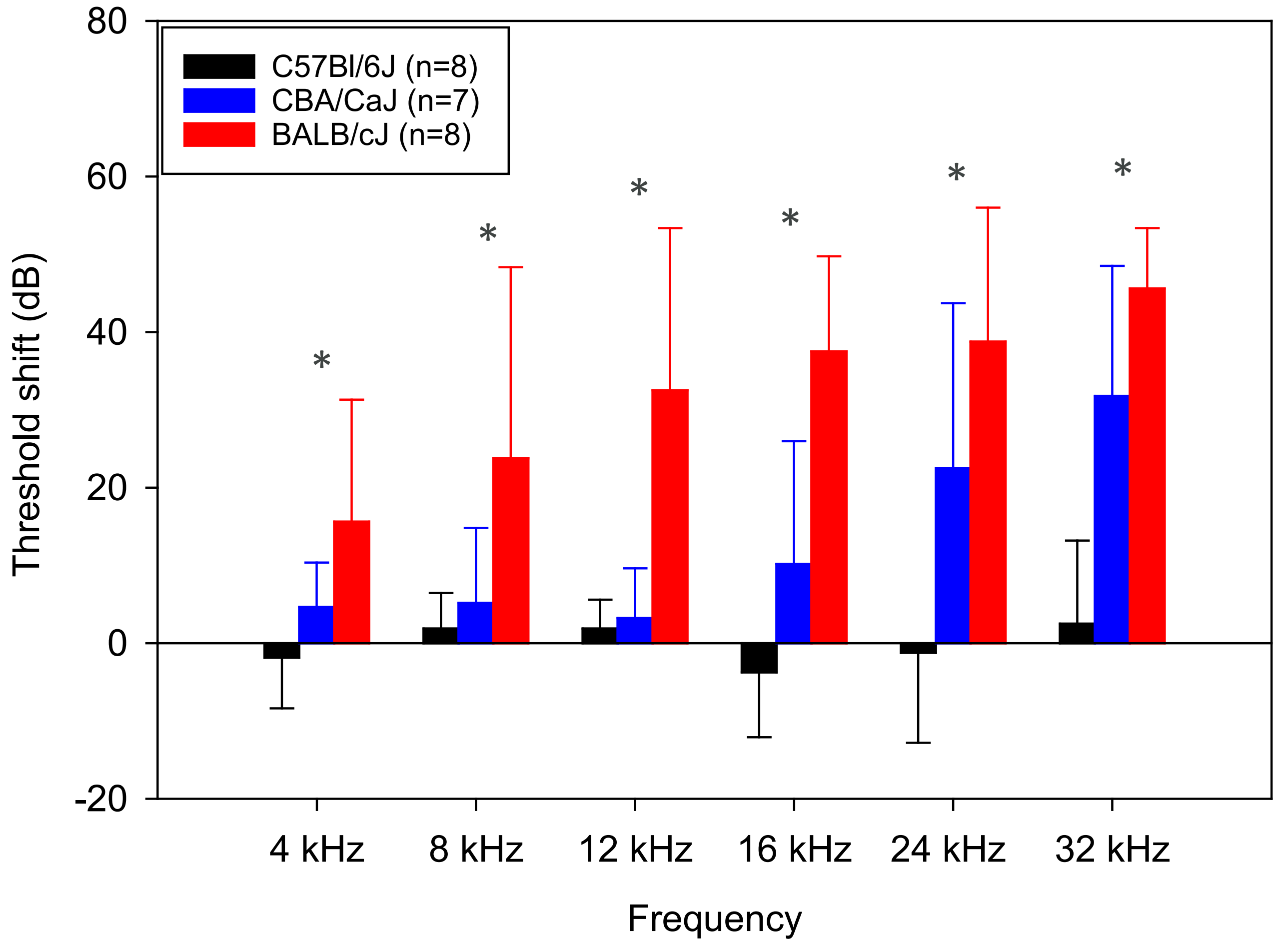
1) C57Bl/6J

2) CBA/CaJ

3) BALB/cJ

Comparison of strains after each round:

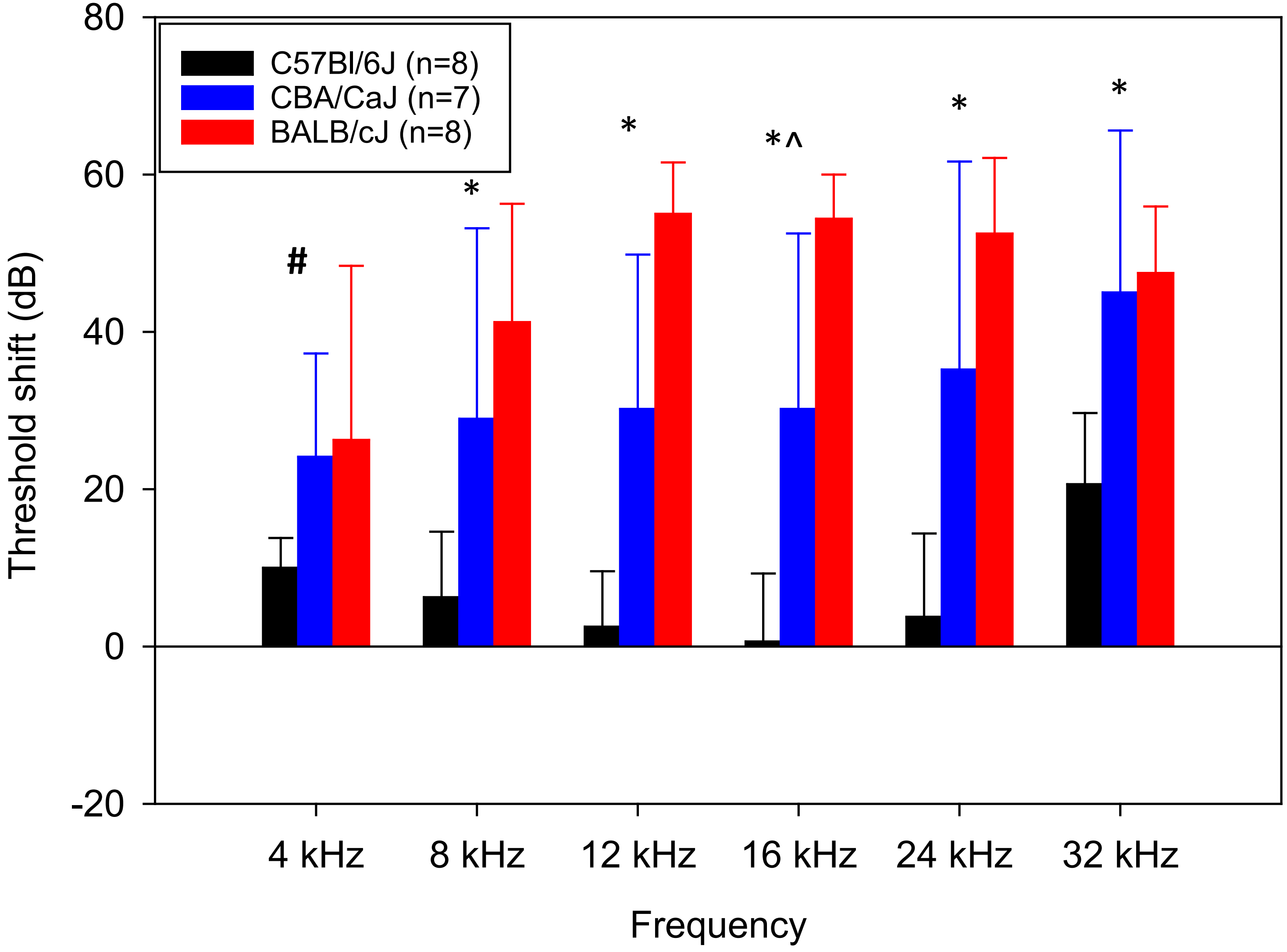
Round 1:
16 mg/kg cumulative



*BALB>C57 and CBA

From DeBacker, Harrison, Bielefeld (2020). *Hearing Research, 387, 107878.*

Round 2:
32 mg/kg cumulative



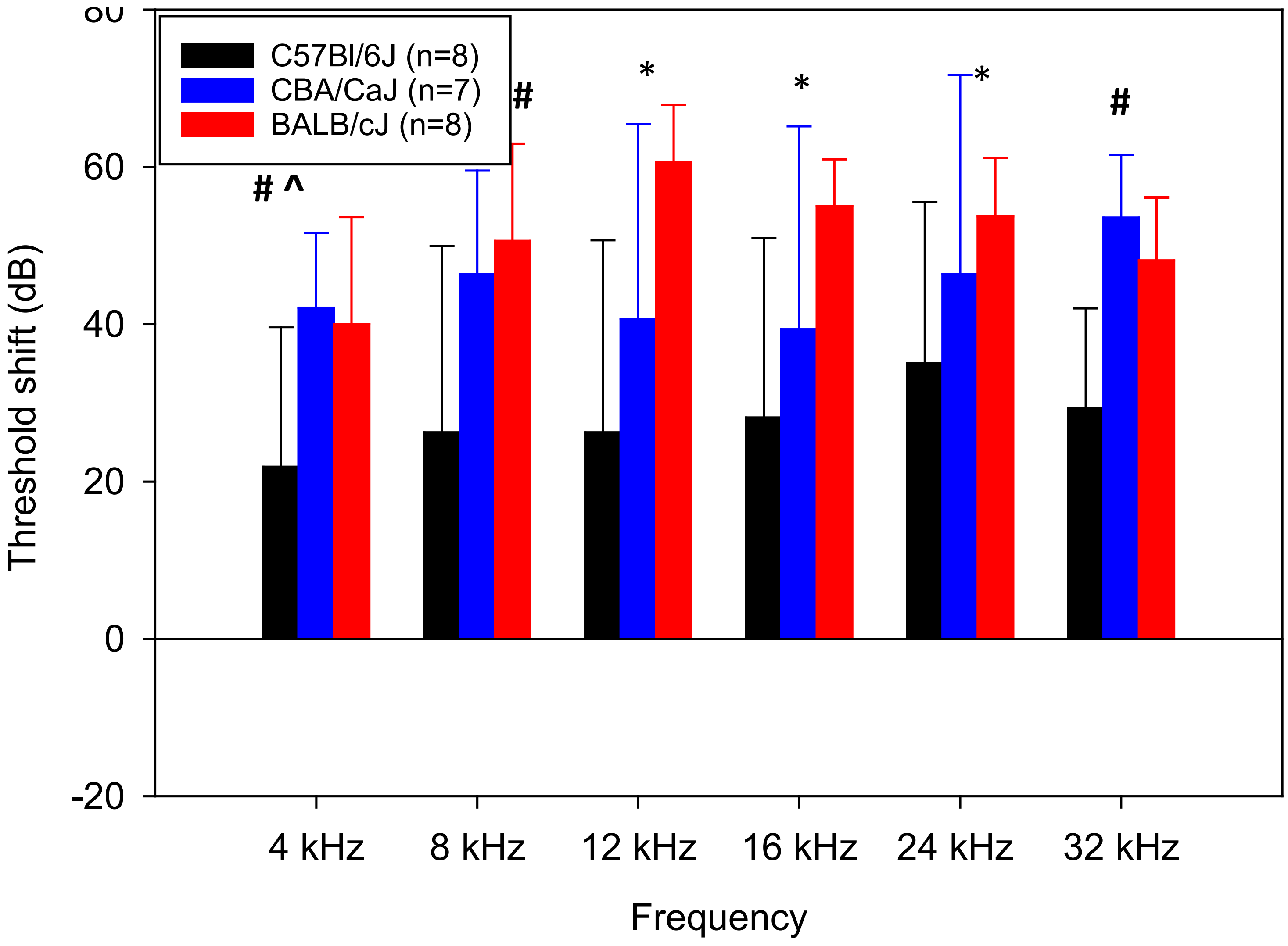
*BALB>C57 and CBA

#BALB>C57 only

^CBA> C57

From DeBacker, et al. (2020)

Round 3:
48 mg/kg cumulative



*BALB>C57 and CBA

#BALB>C57 only

^CBA> C57

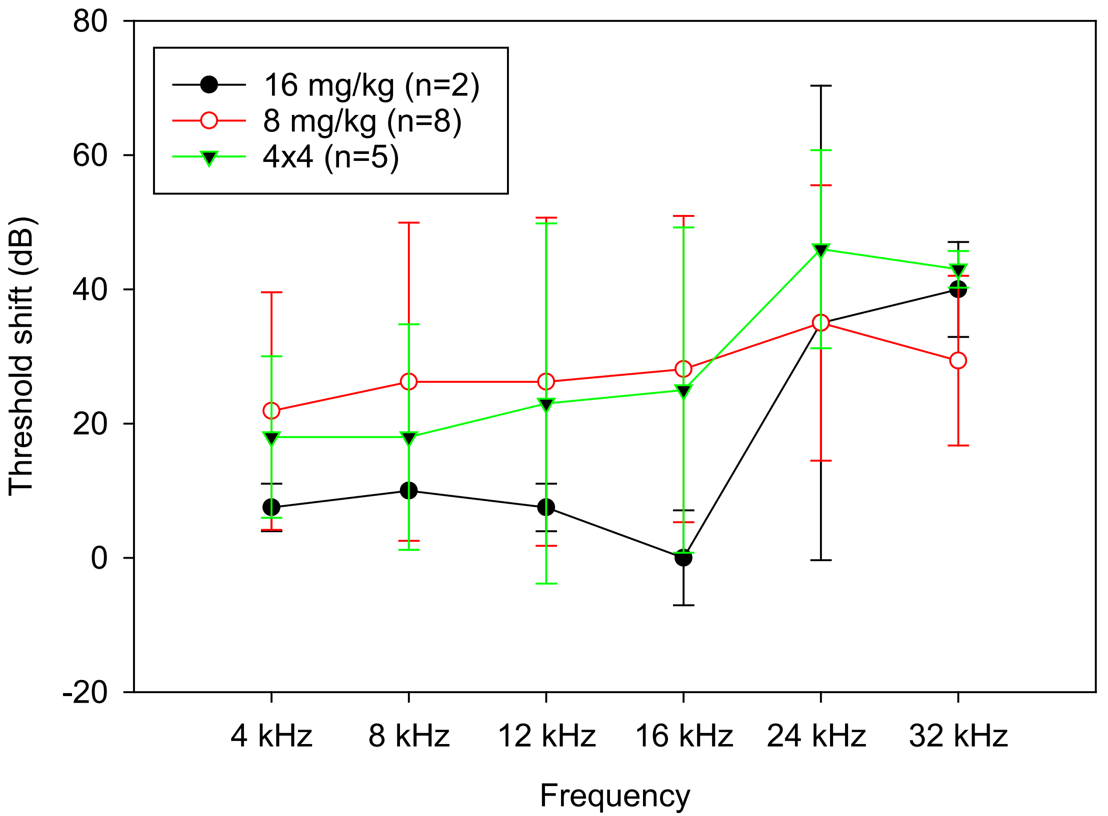
From DeBacker, et al. (2020)

Dose delivery paradigms

- Single 16 mg/kg doses every 3 weeks
- 8 mg/kg doses every 10 days – 2 per 3-week interval
- 4x4: 4 mg/kg daily for 4 consecutive days repeated every 3 weeks

- Total cumulative doses were the same: 48 mg/kg

C57: No differences in delivery on hearing loss - big differences in mortality

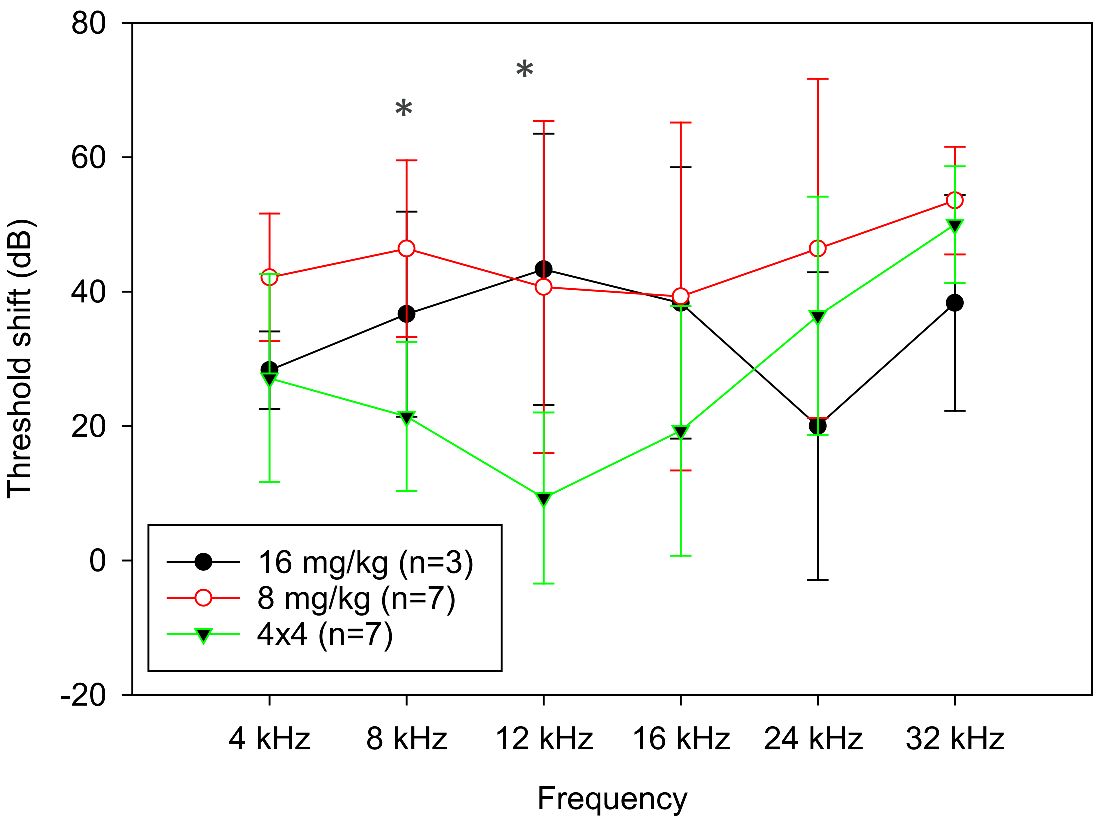


Round 3:
48 mg/kg cumulative

From DeBacker, et al. (2020)

CBA: No differences in delivery on hearing loss after Rd 3 - big differences in mortality

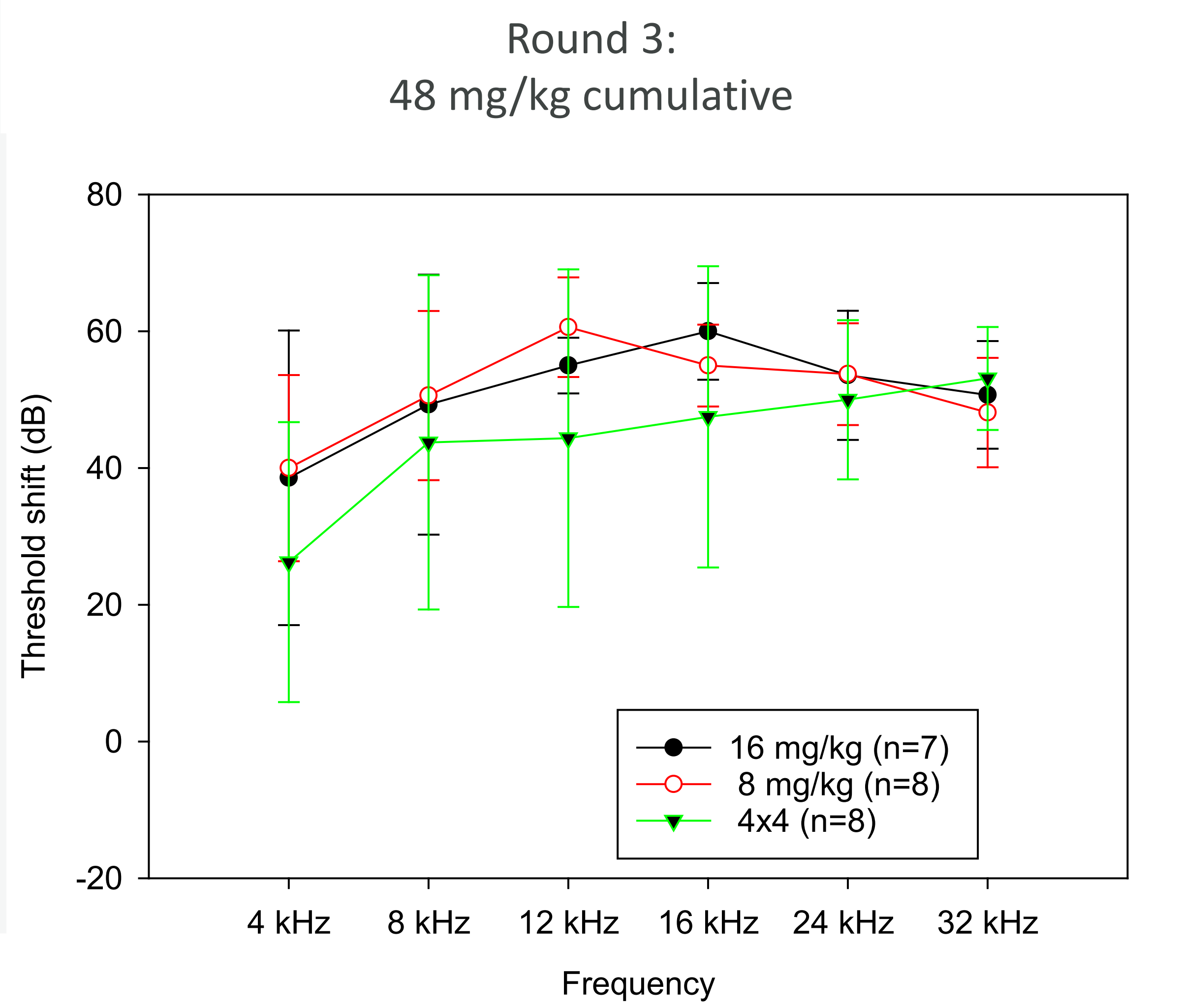
Round 3:
48 mg/kg cumulative



* 8 mg/kg group > 4x4 group

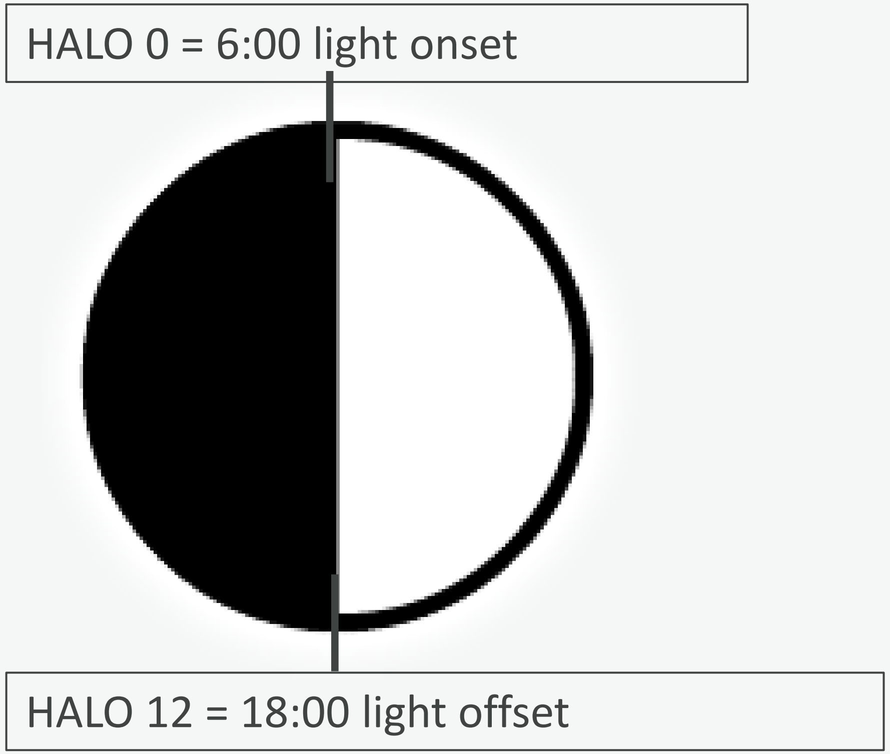
From DeBacker, et al. (2020)

BALB: No differences in delivery on mortality or hearing loss after Rd 3



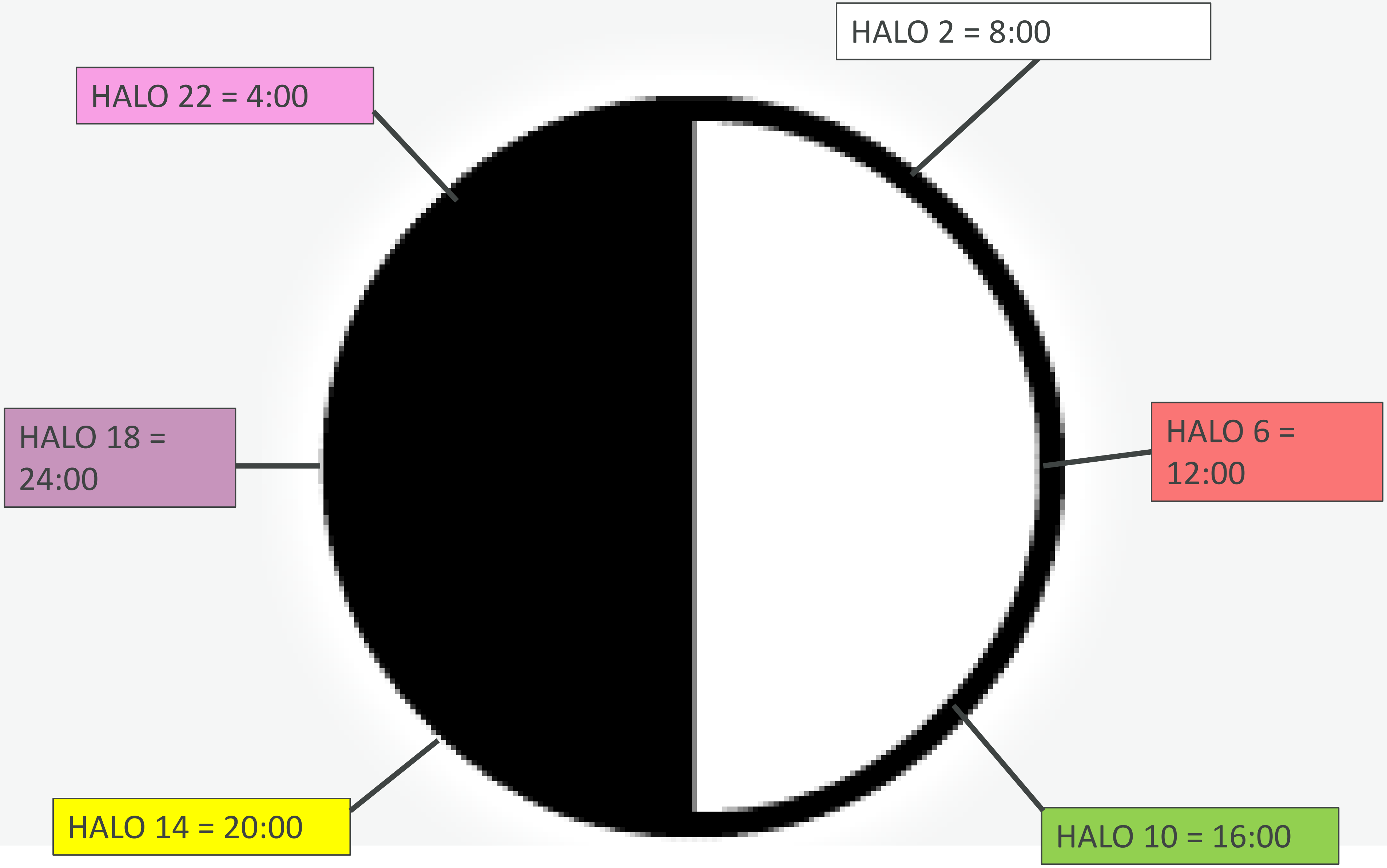
From DeBacker, et al. (2020)

As we expand our definition of ototoxicity, we can also expand options for preventing it

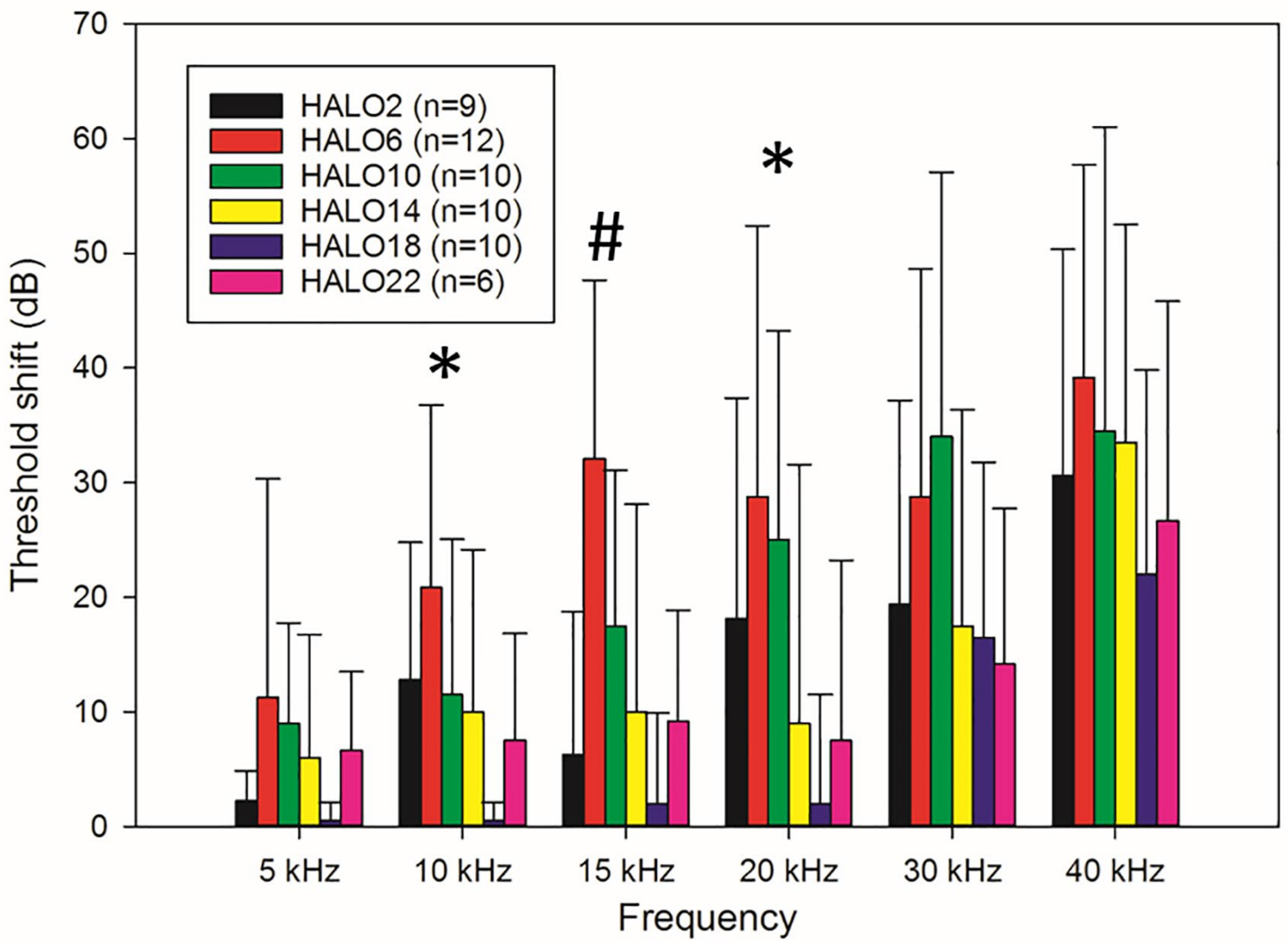


Chronotolerance: differential side effects for a compound based on the time point on the circadian cycle it is delivered

Fischer 344/NHsd rat exposure times

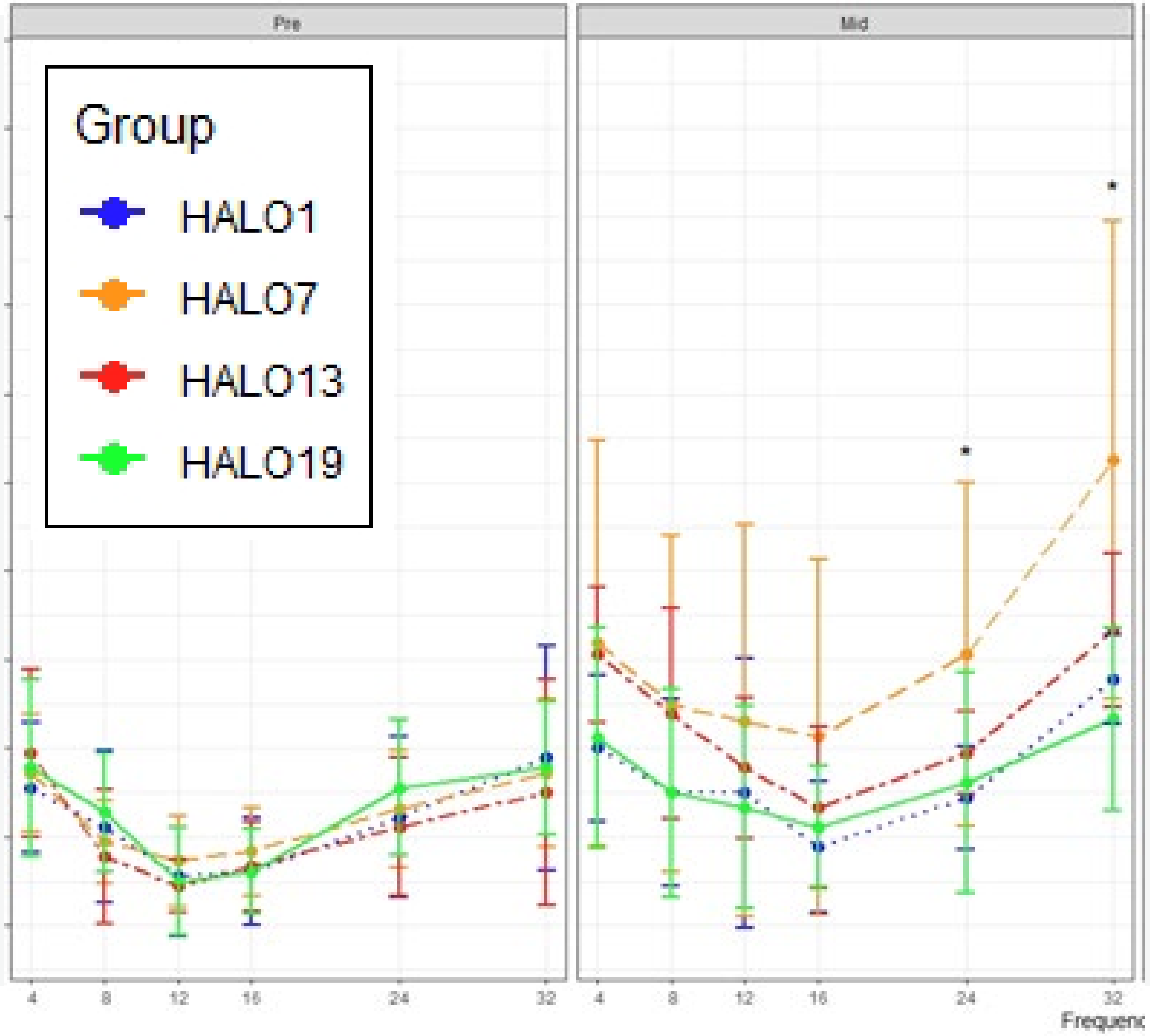


ABR threshold shifts on Day 3



From Bielefeld, Markle, and DeBacker (2018). *Hearing Research*, 370, 16-21.

Mouse study using CBA/CaJ mice and 24 mg/kg cumulative dose over 21 days



From Bielefeld and Harrison. Manuscript submitted

Investigating new classes of compounds for ototoxicity

- Anti-retroviral medications
- Methadone and opioids

Anti-retroviral medications

- ARVs: used to manage HIV infection
- Anecdotal evidence of hearing problems with ARV treatment
- NRTIs: nucleoside reverse transcriptase inhibitors
  - Primarily compounds in most HIV ARV cocktails
  - Can damage the mitochondria by depleting mtDNA

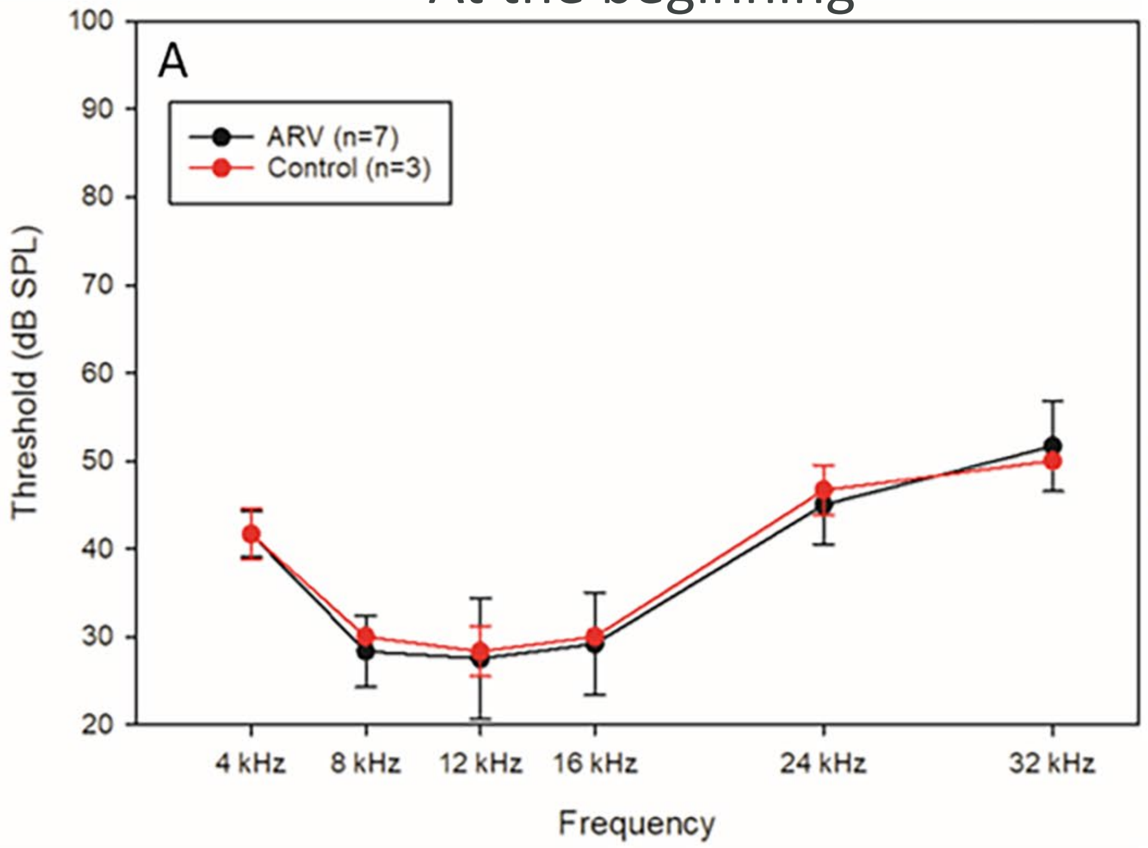
Gestational and nursing exposure to ARVs

- Dosed pregnant/nursing female C57Bl6/J mice with ARVs Lamivudine and Tenofovir by daily oral gavage

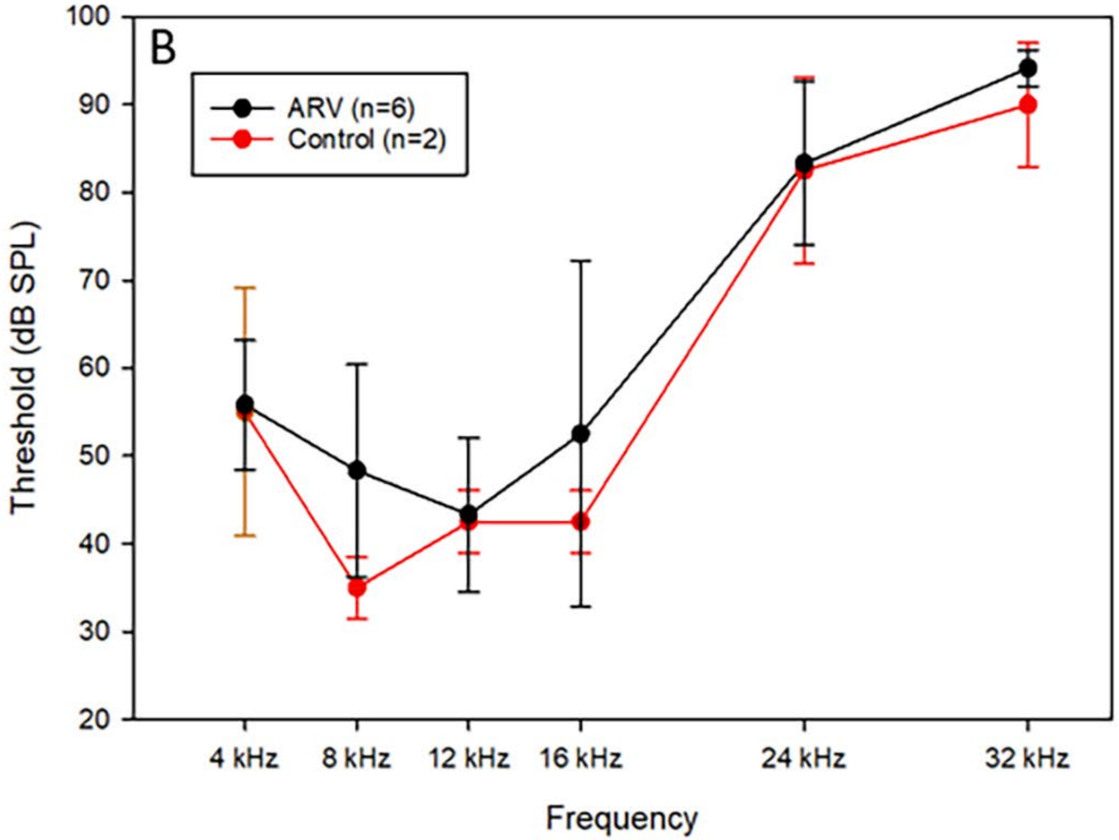
- Assessed ABRs and susceptibility to noise-induced hearing loss in the offspring

Thresholds in the females over duration of the experiment

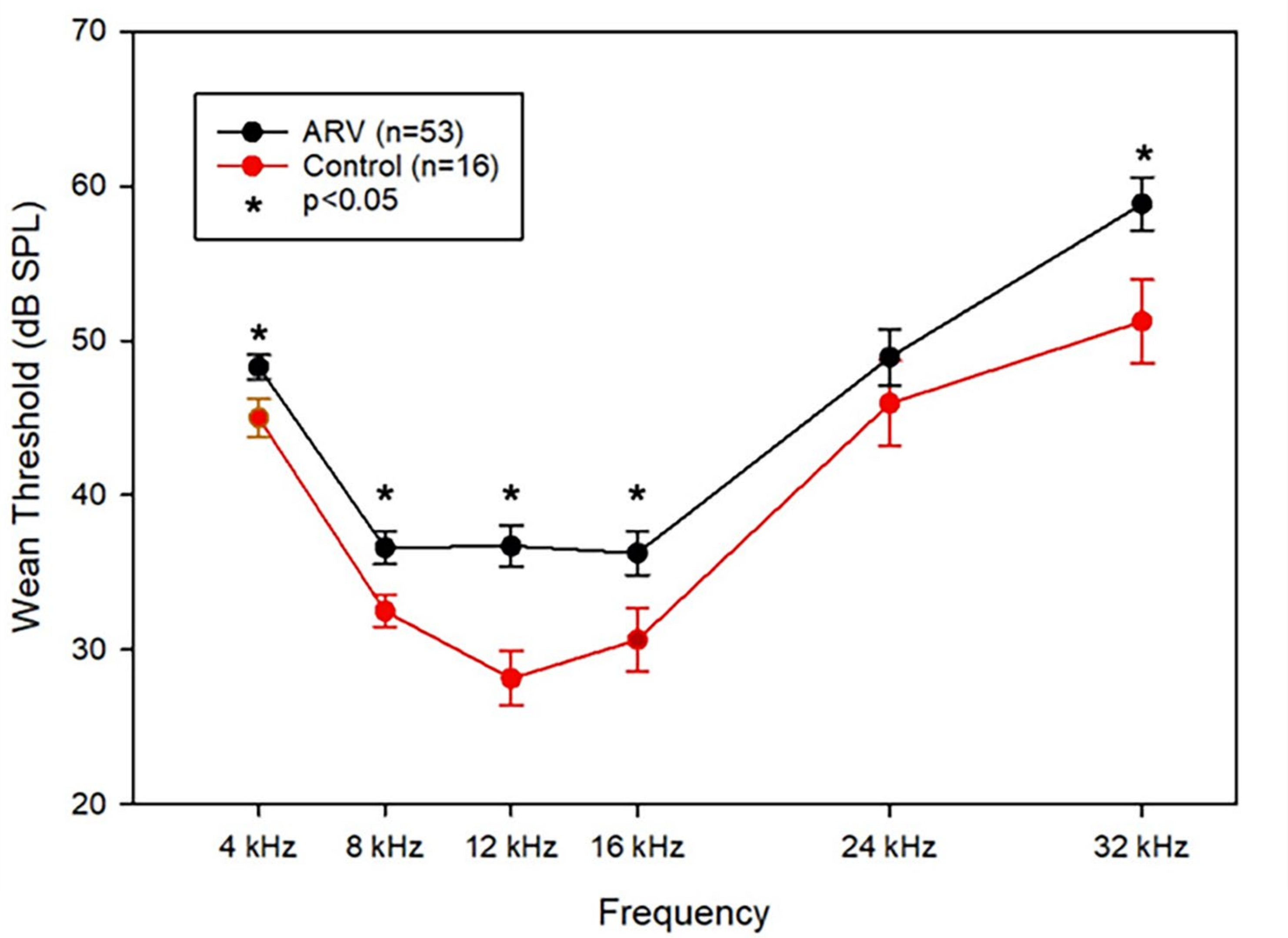
At the beginning



At the end

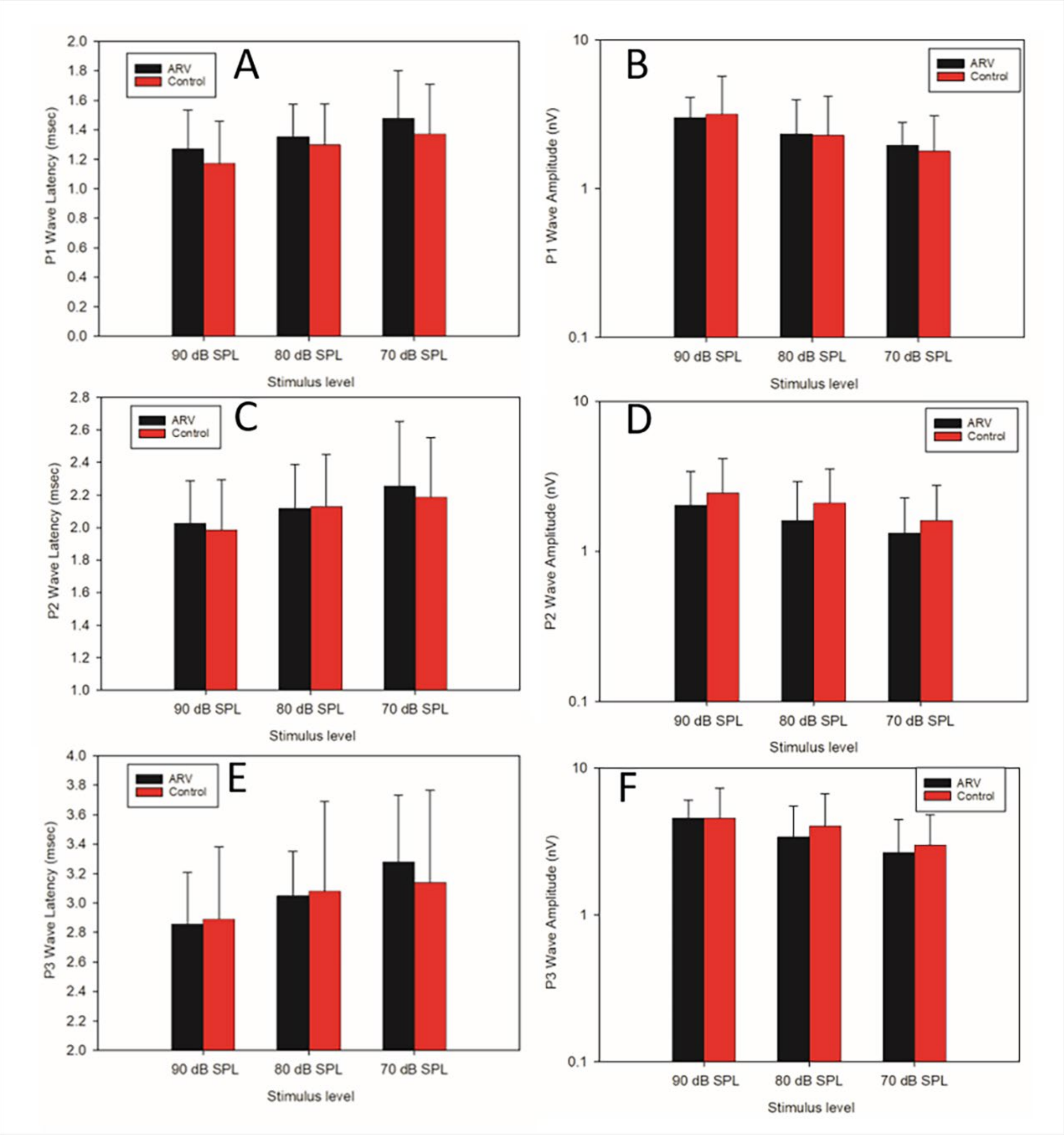


Offspring thresholds at 3-week weaning

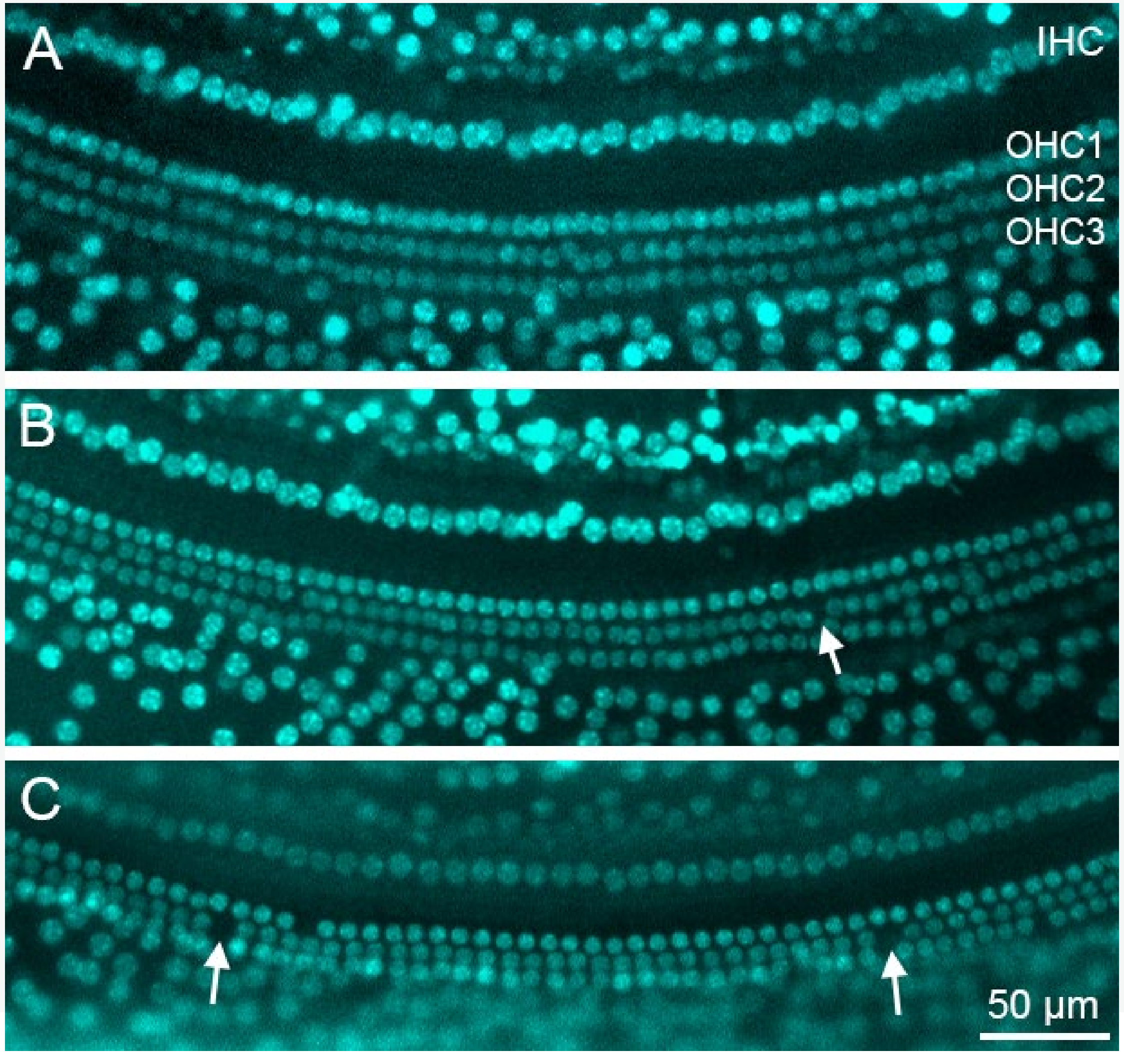


From DeBacker, Hu, and Bielefeld. Manuscript under revision

Offspring supra-threshold ABRs at 3-week weaning



Offspring representative hair cells



Apical turn

Middle turn

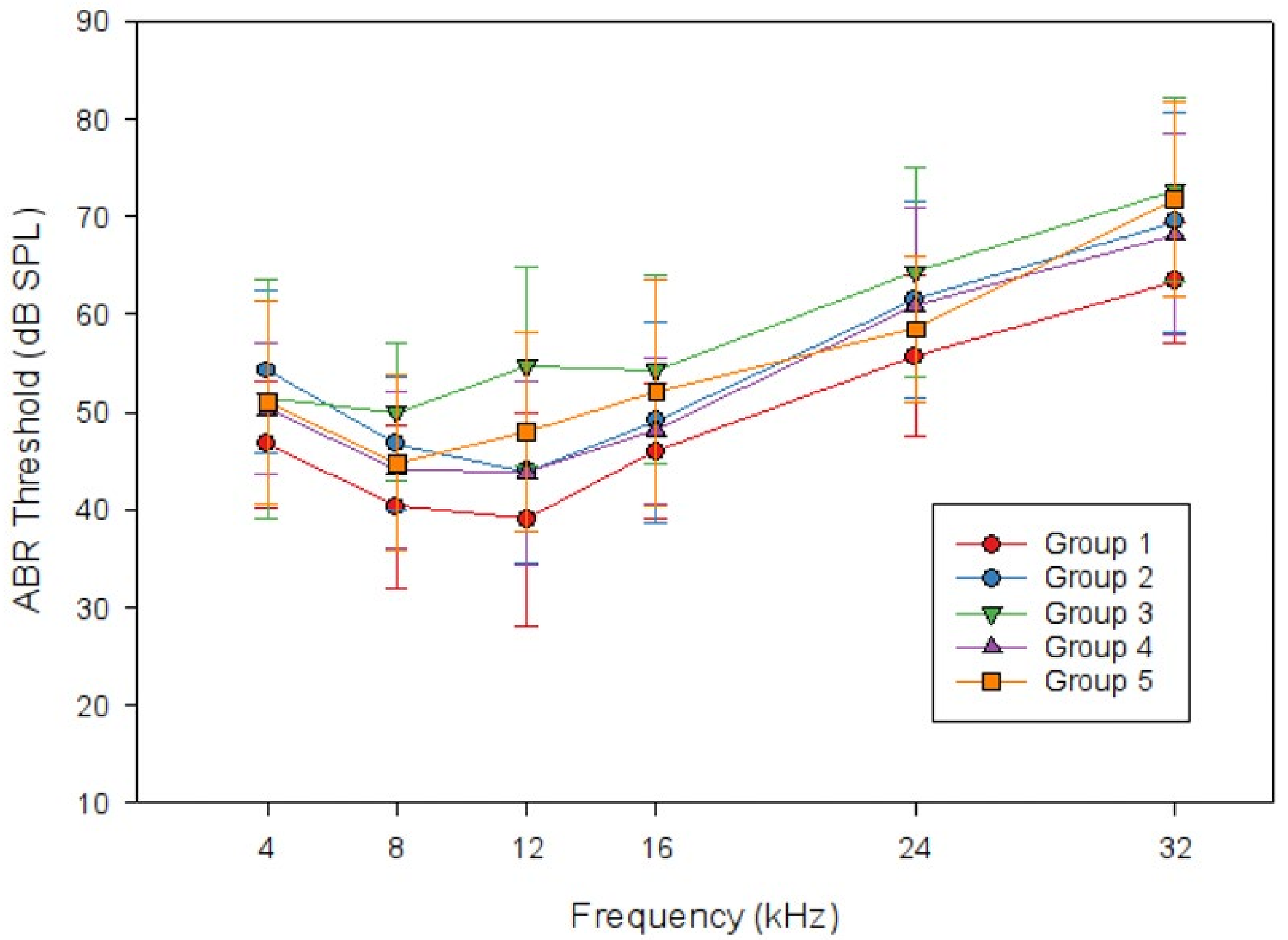
Basal turn

Expanding the roster of NRTIs studied

EXPERIMENTAL GROUPS:

1) Control - water
2) Lamivudine (3TC) + tenofovir (TDF) + efavirenz (EFV)
3) 3TC + zidovudine (AZT) + EFV
4) 3TC + TDF + nevirapine (NVP)
5) 3TC+ AZT + NVP

Offspring thresholds at 3-week weaning



Group 2, 3, 5 > Group 1 at all freqs

Group 2 > 1 by ~5 dB
Group 3 > 1 by ~9 dB
Group 5 > 1 by ~5 dB

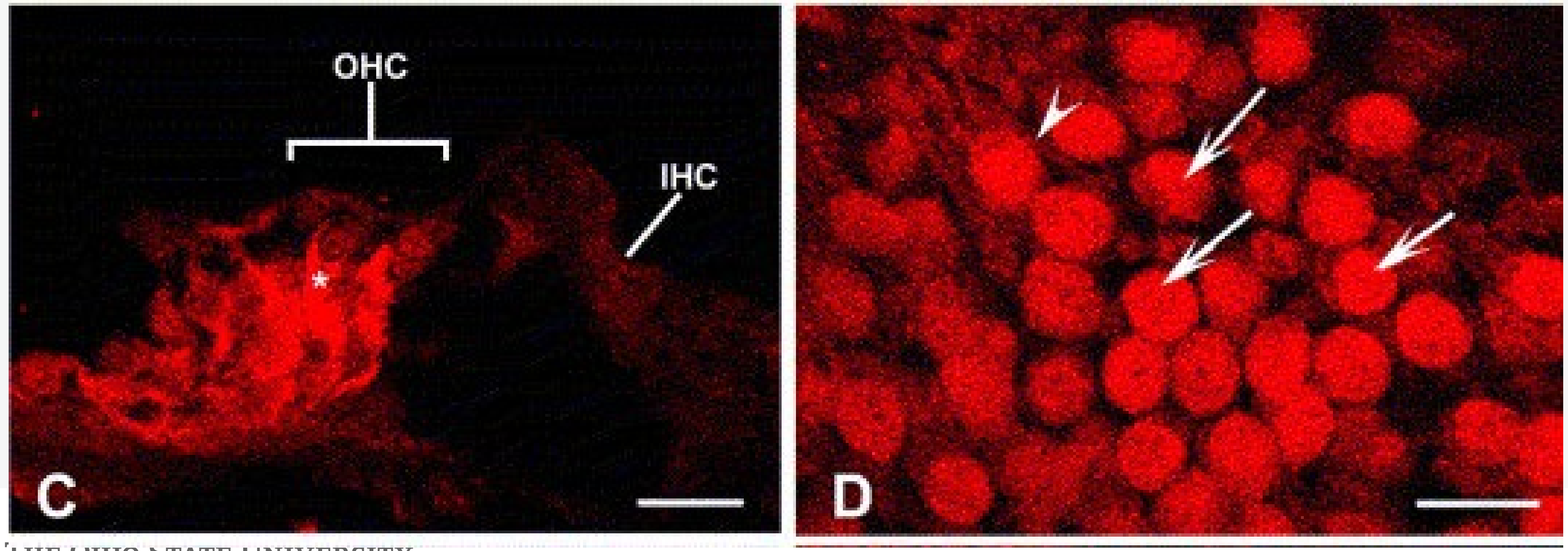
From DeBacker, Langenek, Bielefeld, in preparation

Takeaways

1) Gestational and nursing exposure to ARVs leads to higher mean thresholds in offspring
2) Not accounted for with hair cell losses
3) No physiologic evidence of hidden hearing loss
4) Threshold elevations would not be captured on hearing screening or even full diagnostic ABR test

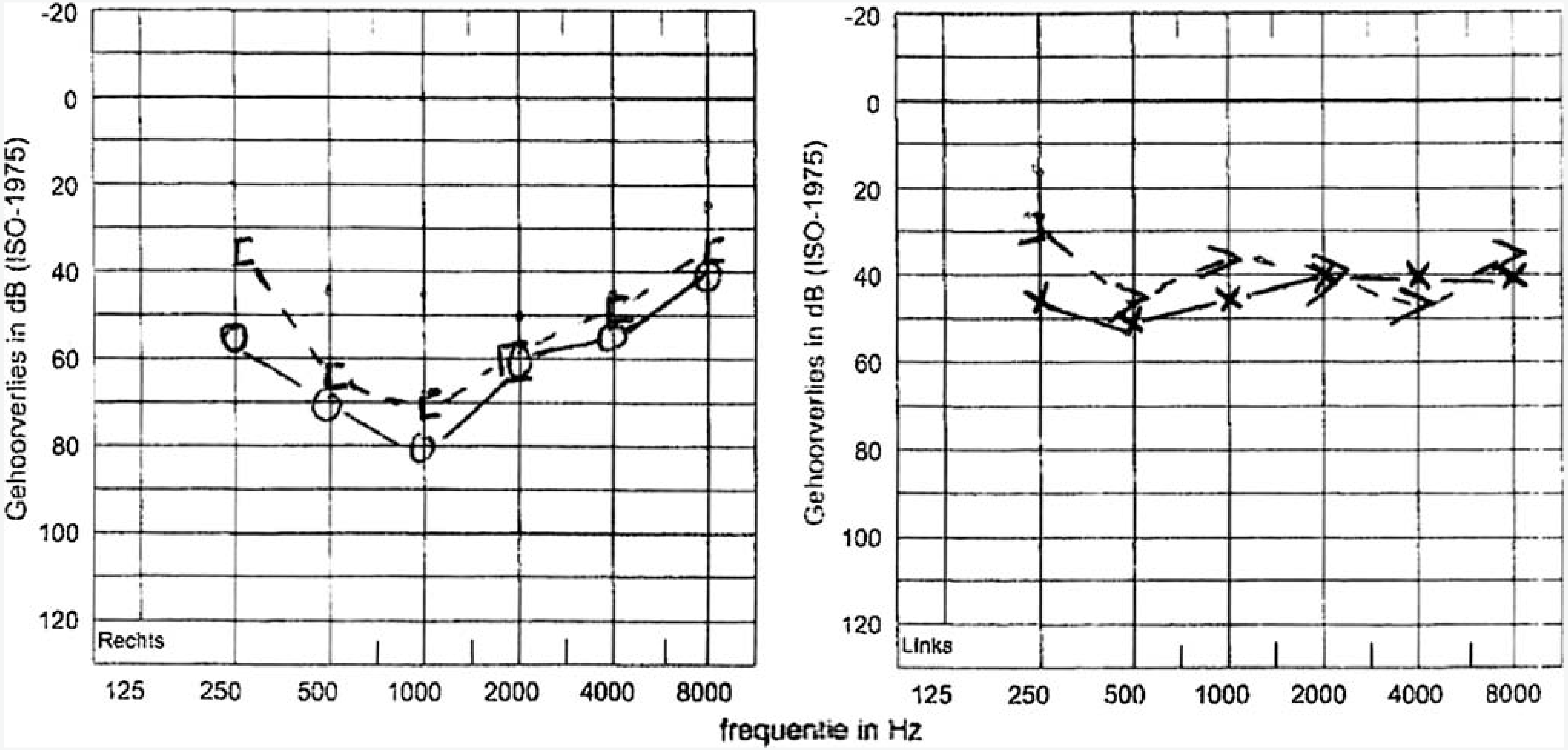
Exploring ototoxicity from Methadone

- Methadone maintenance treatment (MMT) is a common approach to treating opioid use disorder
- Necessitates use of methadone for weeks, months, years
- Acts on mu opioid receptors



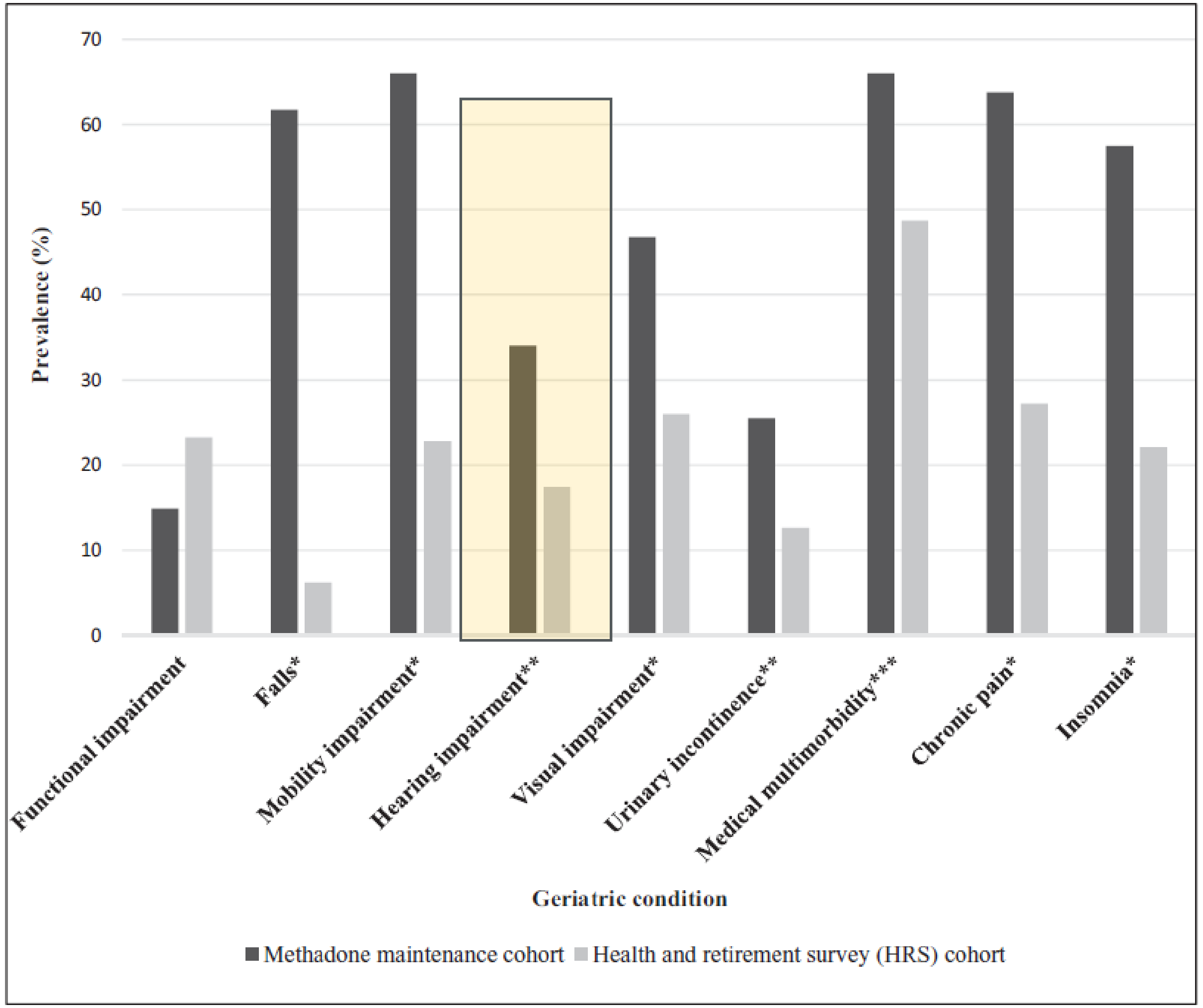
From Jongkamonwiwat et al. 2003, *Hearing Research*, 181, 85-93

Sudden SNHL from Methadone



From van Gaalen et al. 2009, *Eur Arch Otol*

Increased prevalence of hearing loss in adults using MMT



From Han et al. 2020, *J Addiction Med*

This year

1) Oral gavage dosing of CBA/CaJ mice with MMT for 3, 6, 9 months

   -Track with ABRs and OAEs to assess ototoxicity

   -Post mortem hair cell assessments

2) Oral gavage dosing of C57Bl6/J mice for 3 months and then track aging afterward

References Cited

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