

Authors: Coyat C.¹, Baudoux V.¹, Souchal M.¹, Pucheu S.¹, Malmström S.¹, Vincent C.¹, Fontayne L.¹, Peytavy M.¹, Cazals Y.¹, Cazevielle C.², Toomey R. J.³, Quaille M.³ and Naert G.¹
¹CILcare, Montpellier, France; ²Institut des Neurosciences, Université de Montpellier, France; ³GSK, Colledgeville, USA

OBJECTIVES

Occupational and recreational noise-induced hearing loss is a complex disease with increasing global prevalence. Exposure to intense or prolonged sounds has a damaging effect on cochlear structures which can be irreversible. Acoustic trauma can lead to permanent or transitory threshold shifts (PTS and TTS) with different pathological features, including damage to ribbon synapses (synaptopathy), inner and outer hair cells (IHCs & OHCs) loss, degeneration of fibers and SGNs.

The objective of this study was to develop preclinical models in rats to obtain different patterns and severity of NIHL, and gain insight for future pharmacological treatments.

METHODS

Noise Exposure (NE): Animals were anesthetized using Ketamine/Xylazine (75 mg/kg + 10 mg/kg, IP) and submitted to a bilateral NE at a noise band of 8-16 kHz (Open Field) at different intensities and durations.

ABR: Auditory Brainstem Responses (both ears) were measured (eight animals/one ear at a time) 21 days after the NE at 5 frequencies: 4, 8, 16, 25 and 32 kHz. The signals were amplified (gain 10 000, band pass 100-5000 Hz). The stimuli consisted of tone pips (1 ms linear rise/fall time) presented in 10 dB steps from 90 to 0 dB.

DPOAE: Distortion Product Otoacoustic Emissions at T_{+21DAYS} were assessed (one animal/one ear at a time) at 4, 8, 16, 24 and 32 kHz at an intensity of 63 dB.

Cochleograms and synaptic ribbons: At T_{+21DAYS}, the cochleae were removed, perfused with PFA 4%, fixed at RT for 1h in PFA 4%, dissected (no decalcification) and immunostained.

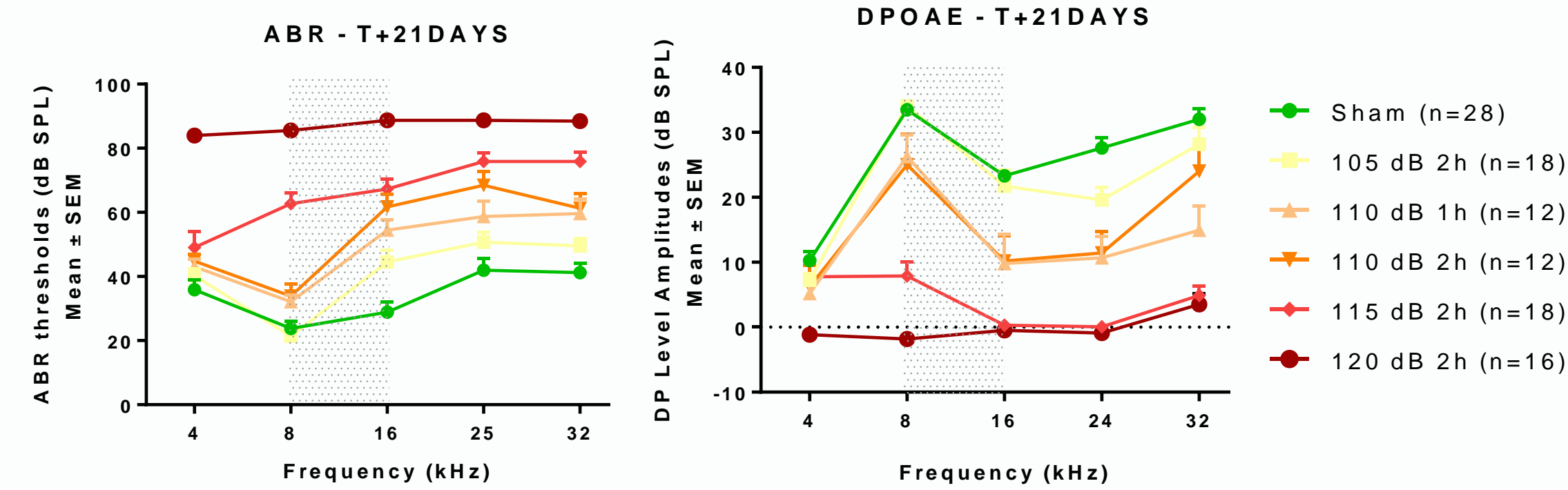
Fibers and SGN: At T_{+35DAYS}, the cochleae were sampled, fixed O/N in Glutaraldehyde 2.5%, decalcified over 7 days in EDTA 10%, post fixed in osmic acid 2% (1h), dehydrated in baths of ethanol (from 30 to 100%) and embedded in a resin. The cochleae were cut (1 μm thick) and stained with toluidine blue.

CONCLUSION

By varying the intensity of the NE by 5 dB steps and modifying the duration, different phenotypes of HL were obtained, from slight functional impairment with only a loss of synaptic ribbons and no HC loss to significant HL with strong degeneration of hair cells, fibers and SGN. These five models allow to mimic different aspects of NIHL, separately or combined. This complete characterization of each NIHL model can support the efficacy testing of drugs targeting different pathways: synaptogenesis, cell death (apoptosis or necrosis), oxidative stress, inflammatory responses, and/or cell regeneration.

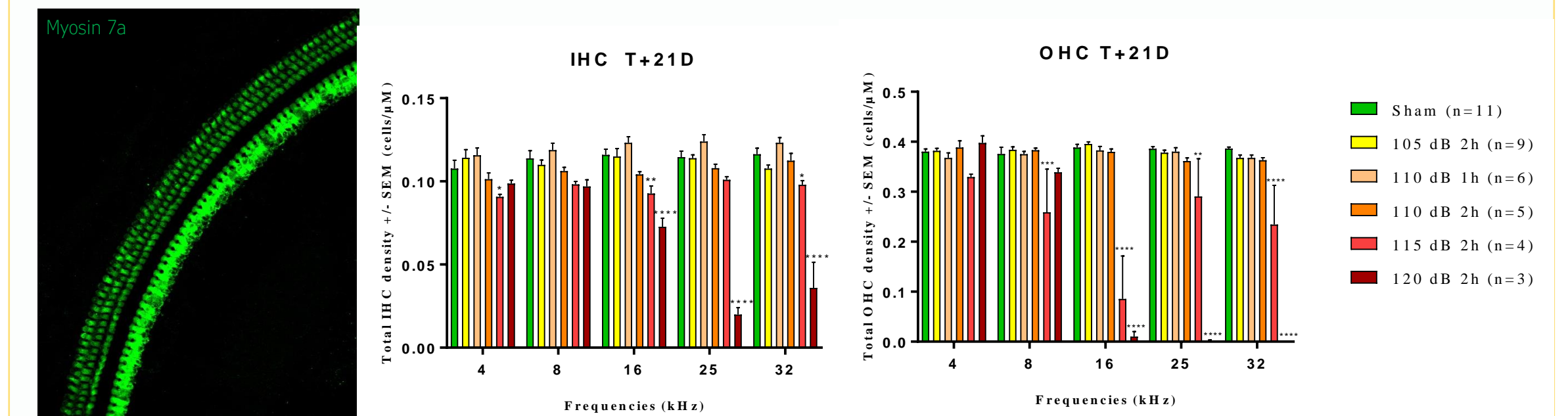
RESULTS

AUDITORY FUNCTION



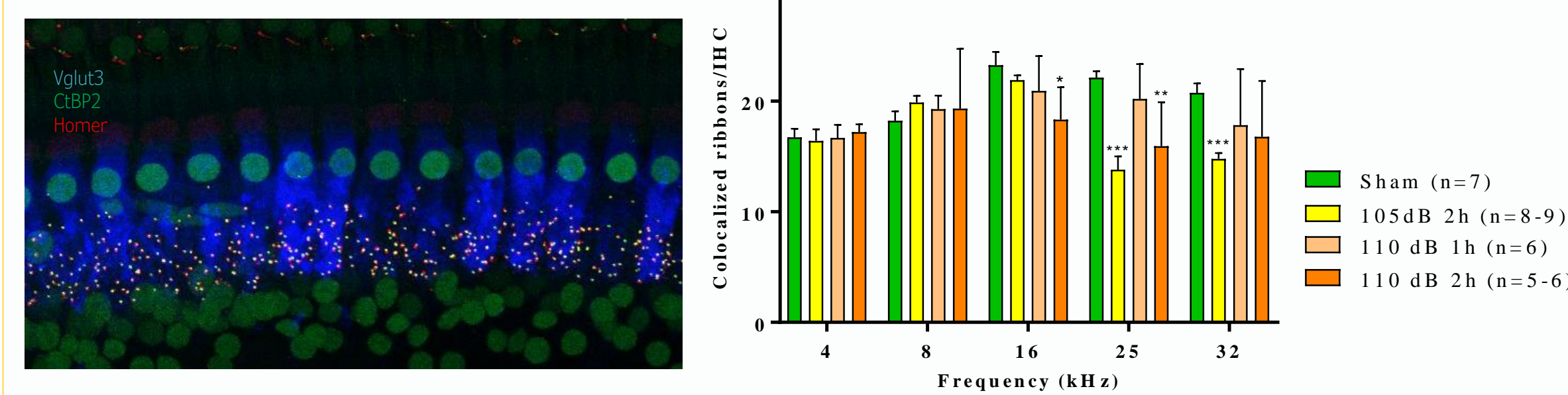
The results showed a good correlation between the increase of the noise intensity and exposure and the hearing loss (HL) severity, demonstrated by a progressive increase in ABR thresholds and a progressive decrease in DPOAE amplitudes from 105 dB to 120 dB NE.

COCHLEOGRAM



The number of IHCs and OHCs (Myosin 7A⁺ cells) was counted at 4, 8, 16, 25 and 32 kHz 21 days after NE. No hair cell loss occurred after NE at 105 and 110 dB (1 h or 2 h). IHC and OHC loss was observed only for greater intensity and duration: NE at 115 dB (2h) induced IHC loss at 16 and 32 kHz and OHC loss from 8 kHz; at 120 dB, a more important loss of IHC and OHC from 16 kHz.

SYNAPTIC RIBBON



The colocalized punctae of CtBP2 and Homer were counted at 4, 8, 16, 25 and 32 kHz at T_{+21DAYS} for the animals exposed at 105 and 110 dB. No count was done on the 115 and 120 dB groups. Significant loss of synaptic ribbons was observed for NE at 105 dB (1h) at 16 and 32 kHz and for NE at 110 dB (2h) at 16, 25 and 32 kHz. NE at 110 dB (1h) induced no synaptic loss.

FIBER & SGN

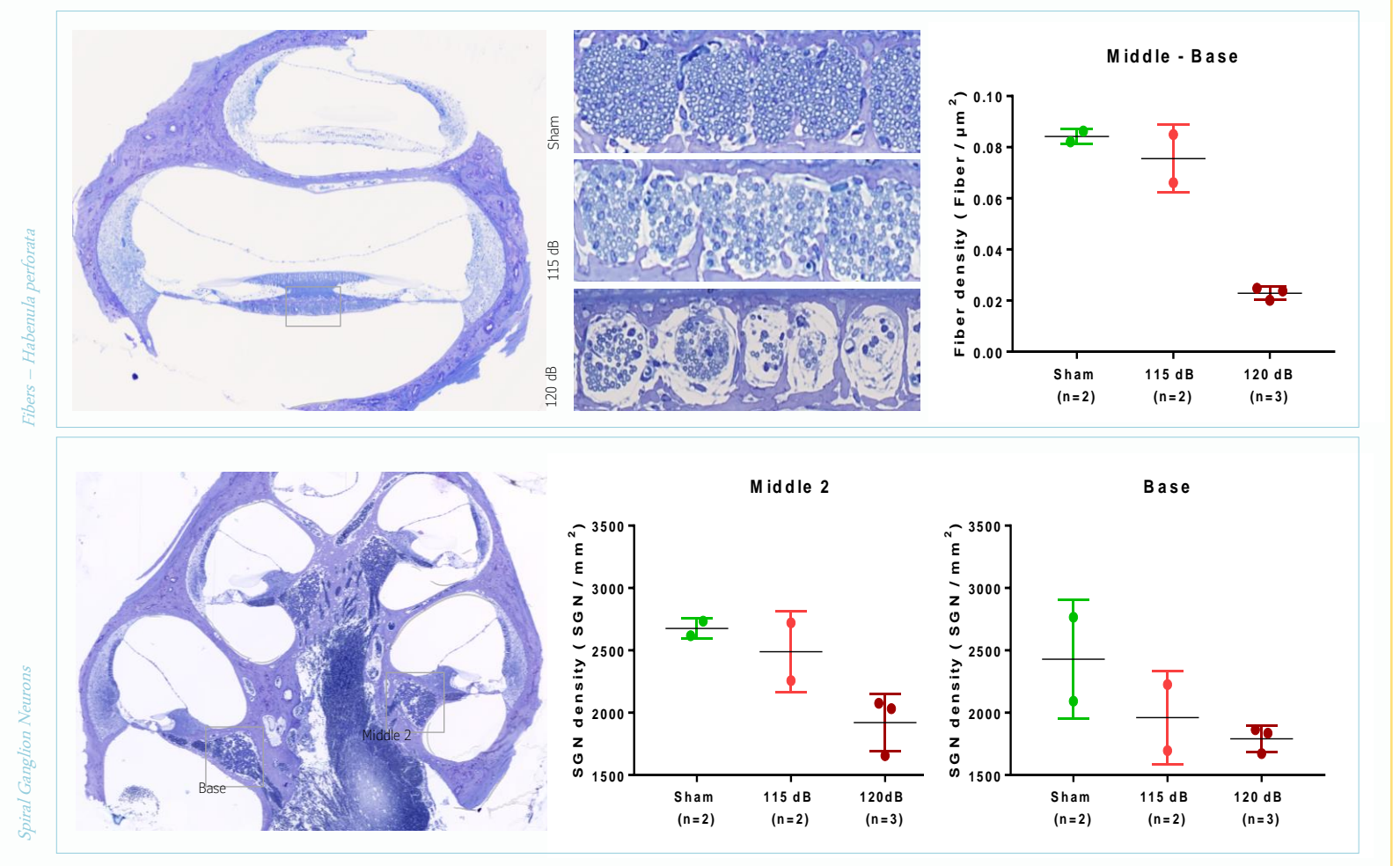
Preliminary results:

115 dB group:

1/2 cochlea showed a slight decrease of fibers and SGN on the basal turn (<20%)

120 dB group:

3/3 cochleae showed severe loss of fibers (73%) and a decrease of SGNs (<30%).



NIHL in male Wistar rats	Noise Trauma	ABR threshold Shift (dB) vs BL				DPOAE amplitude shift (dB) vs BL				Cochleograms (IHC / OHC - % loss)				Synaptic Ribbons (% loss)			Fiber (% loss)			SGN (% loss)			Conclusion	
		Intensity	Duration	Freq	Range	8 kHz	16 kHz	25 kHz	32 kHz	8 kHz	16 kHz	24 kHz	32 kHz	8 kHz	16 kHz	25 kHz	32 kHz	16 kHz	25 kHz	32 kHz	Mid/Base	Middle		Base
	Sham (not traumatized)	6	5	4	2	3	4	1	5	For each parameter, the values of the Sham group (mean=100%) are used as reference												Sham, normal HC number		
	105 dB 2h 8-16 kHz	4	15	8	7	3	-1	-4	0	3 / -2	1 / -2	1 / 2	7 / 5	6	38	29	NOT PERFORMED			TTS, no HC loss, synaptopathy				
	110 dB 1h 8-16 kHz	15	21	15	18	-5	-14	-14	-17	-4 / 0	-6 / 2	-8 / 1	-6 / 5	10	9	14	NOT PERFORMED			Slight PTS, no HC loss, weak synaptopathy				
	110 dB 2h 8-16 kHz	17	30	25	23	-6	-11	-14	-8	7 / -2	10 / 2	6 / 6	3 / 6	21	28	19	NOT PERFORMED			PTS, rare OHC loss, synaptopathy				
	115 dB 2h 8-16 kHz	37	48	39	35	-20	-15	-24	-18	14 / 31	20 / 78	12 / 25	16 / 39	NOT PERFORMED			10	7	19	PTS, HC loss, minor fiber & SGN loss				
	120 dB 2h 8-16 kHz	60	64	50	45	-32	-20	-30	-21	15 / 10	37 / 97	83 / 100	69 / 100	NOT PERFORMED			73	28	26	PTS, severe loss of HC & fibers, loss of SGNs				