DEVELOPMENT OF NOISE-INDUCED HEARING LOSS (NIHL) WISTAR RAT MODELS FOR PRECLINICAL EFFICACY ASSESSMENT

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OBJECTIVES

Occupational and recreational noise-induced hearing loss is a complex disease with an 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: The 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.

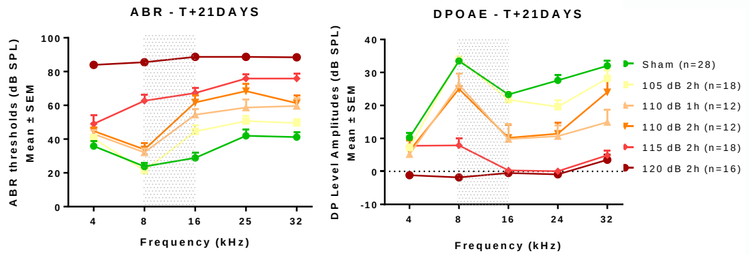
Cochleograms and synaptic ribbons: At $T_{+21DAYS}$, the cochleae were removed, perfused with PFA 4%,fixed at RT during 1h in PFA 4%, dissected (no decalcification) and immunostained.

Fibers and SGN At $T_{+35DAYS}$, the cochleae were sampled, fixed O/N in Glutaraldehyde 2.5%, decalcified 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.

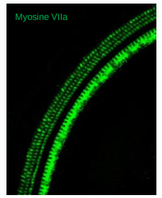
RESULTS

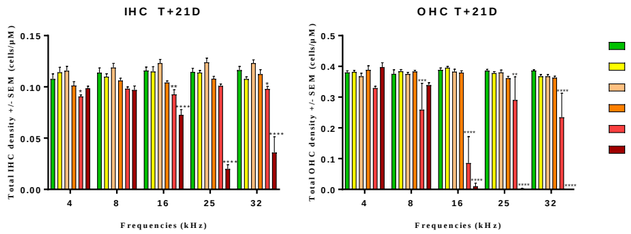
Auditory function

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.



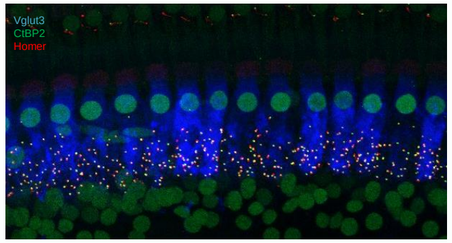
Cochleograms

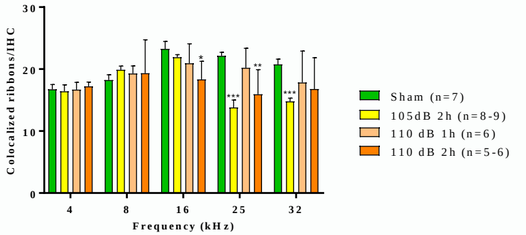




The number of IHCs and OHCs (Myosin 7A+ cells) was counted at 4, 8, 16, 25 and 32 kHz 21 days after NE.

Synaptic ribbon





The colocalized punctae of CtBP2 (BD Biosciences- 612044-1/500) and Homer (GeneTex-GTX103278-1/500) 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.

Fiber & SGN

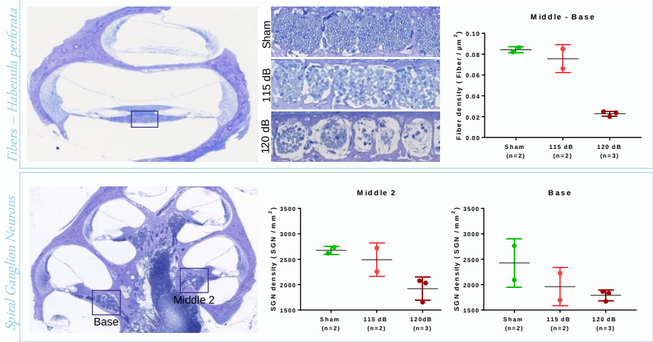
Preliminary results :

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	Functional/morphological/cellular characterization
	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, no to weak 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 and fibers, loss of SGNs

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**.