# Using Virtual Reality to Quantify Cochlear Synaptopathy in Adult & Juvenile Mice after Acoustic Trauma

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## Objectives

- Quantify synaptopathy of adult mice exposed to standard acoustic trauma (110 dB for 2 hours)
- Characterize synaptopathy of juvenile mice after noise exposure (120 dB for 5 minutes)
- Compare hearing loss and synaptopathy in adult and juvenile mice

## Introduction

Previous studies with adult mice have shown that prolonged exposure to loud sound (acoustic trauma) elevates hearing thresholds as measured by Auditory Brainstem Response (ABR) and causes a loss of inner hair cell (IHC) ribbon synapses (synaptopathy)[1]. In young, P18, C57BL/6J (BL-6) mice, a much briefer noise exposure (120 dB for only 5 minutes) causes hearing loss (Saunders and Chen, 1982)[3]. In this study, we compare adult (over P42) and juvenile (P18) mice after damaging noise exposure to ask if synaptopathy (as quantified by CtBP2/ribeye immunolabeling) also occurs in young mice during this early critical period.

**Figure 1.** Cochlear frequency map

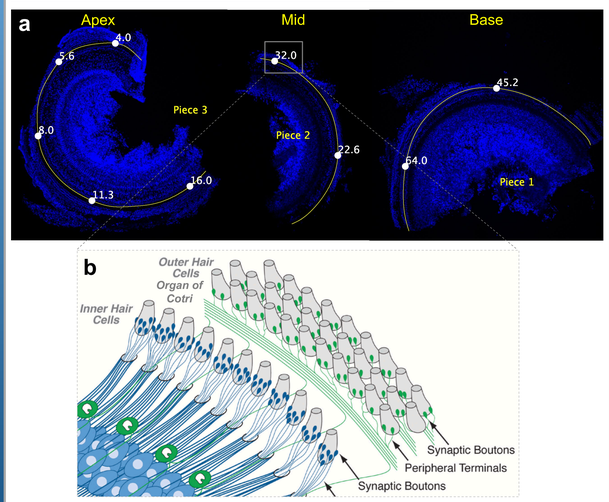


**Figure 1.** a) Frequency map of an adult cochlea. Frequencies marked along the yellow line following the organ of Corti. b) Representative figure of whole mount preparation of the organ of Corti adapted from Liberman (2017)[2].

## Results

**Figure 2.** ABR Waveforms

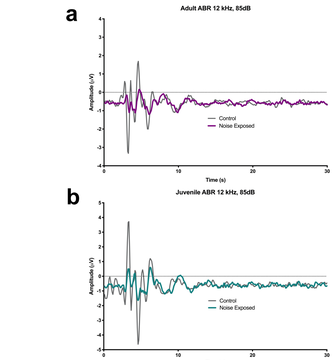


**Figure 2.** Examples of ABR waveforms from control and noise exposed adult (a) and juvenile (b) mice.

**Figure 3.** Acoustic trauma elevates ABR thresholds

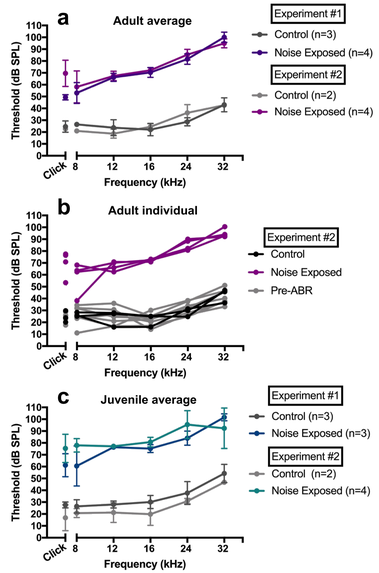


**Figure 3.** a) Average ABR thresholds from two experiments 7 days after acoustic trauma in adult mice. b) Individual thresholds for each adult mouse in the 2nd experiment before noise exposure. c) Average ABR thresholds from two experiments 7 days after acoustic trauma in juvenile mice.

**Figure 4.** Adult mice show significant synaptopathy after acoustic trauma

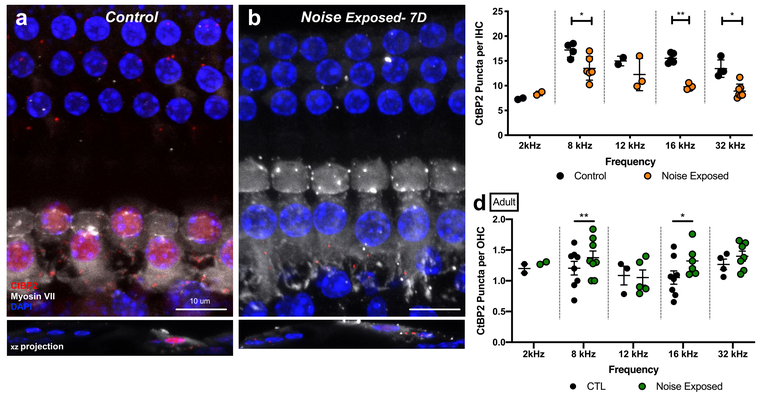


**Figure 4.** a) Representative projection image of the 32kHz region of the adult cochlea from a control (a) and noise exposed (b) mouse. Hair cells were stained with Myosin VII (white), ribbon synapses with CtBP2 (red), and nuclei with DAPI (blue). syGlass software was used to generate videos of these images. c) Quantification of CtBP2 puncta per inner hair cell (IHC) at the indicated frequencies. Each point represents ~ 9 IHC from different cochlea. d) Quantification of CtBP2 puncta under the nucleus per surviving outer hair cell (OHC) at the indicated frequencies. Each point represents 25-30 OHC from different cochlea.

**Figure 5.** Juvenile mice show variability in synapse count after acoustic trauma

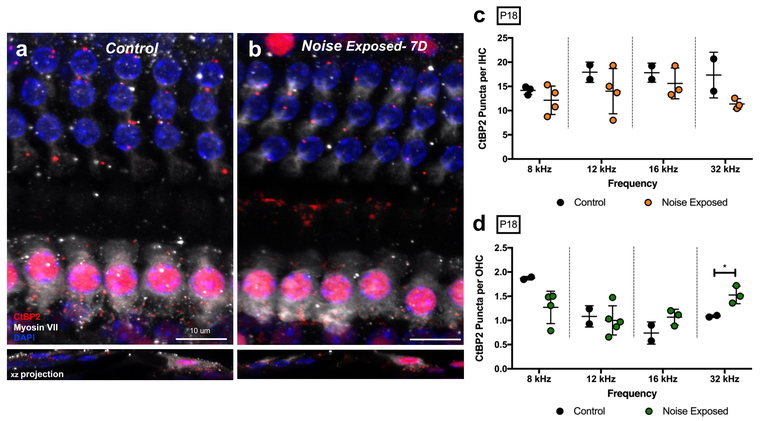


**Figure 5.** a) Representative projection image of the 32kHz region of the juvenile cochlea from a control (a) and noise exposed (b) mouse. Hair cells were stained with Myosin VII (white), ribbon synapses with CtBP2 (red), and nuclei with DAPI (blue). syGlass software was used to generate videos of these images. c) Quantification of CtBP2 puncta per inner hair cell (IHC) at the indicated frequencies. Each point represents ~ 9 IHC from different cochlea. d) Quantification of CtBP2 puncta under the nucleus per surviving outer hair cell (OHC) at the indicated frequencies. Each point represents 25-30 OHC from different cochlea.

## Conclusion

- Adult mice following acoustic trauma have elevated hearing thresholds correlated with loss of synapses in the IHC.
- Juvenile mice have elevated thresholds but more variability in their ABR and synapse quantification. Adjustments to noise exposure protocol have resulted in less ABR variability and synapse quantification is forthcoming.
- The trend of IHC synaptopathy shows similar patterns in both adult and juvenile mice after acoustic trauma.
- The trend of OHCs ribbon synapse increase in number shows similar patterns in both adult and juvenile mice after acoustic trauma.

## References

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[2] Liberman MC. 2017. Noise-induced and age-related hearing loss: new perspectives and potential therapies. F1000Research 6(F1000 Faculty Rev):927. doi: 10.12688/f1000research.11310.1

[3] Saunders JC and Chen CS. 1982. Sensitive periods of susceptibility to auditory trauma in mammals. Environmental Health Perspectives 44(1): 63-66.

## Acknowledgments

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