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# Identifying Novel Molecules Targeting BKCa Channels As Treatment For Auditory Impairments in a Mouse Model of Williams-Beuren Syndrome

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## INTRODUCTION

- ❖ The auditory pathway conveys the special sense of hearing. Sound waves are converted into electrical signals by cochlear hair cells in the inner ear and the auditory nerve carries electrical information to the central nervous system.
- ❖ BKCa channels are key regulators of the electrical activity in the auditory pathway. They regulate neuronal excitability, neurotransmitter release and tuning of cochlear hair cells. BKCa dysfunction can lead to auditory impairments, at both peripheral and central level.
- ❖ Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder caused by a deletion mutation on chromosome 7 and characterized by intellectual disability, distinctive facial features and cardiovascular problems.
- ❖ Reduced BKCa expression has been reported in WBS and auditory impairments, such as mild-to-moderate hearing loss and hyperacusis, are very frequent in WB patients.

### AIMS

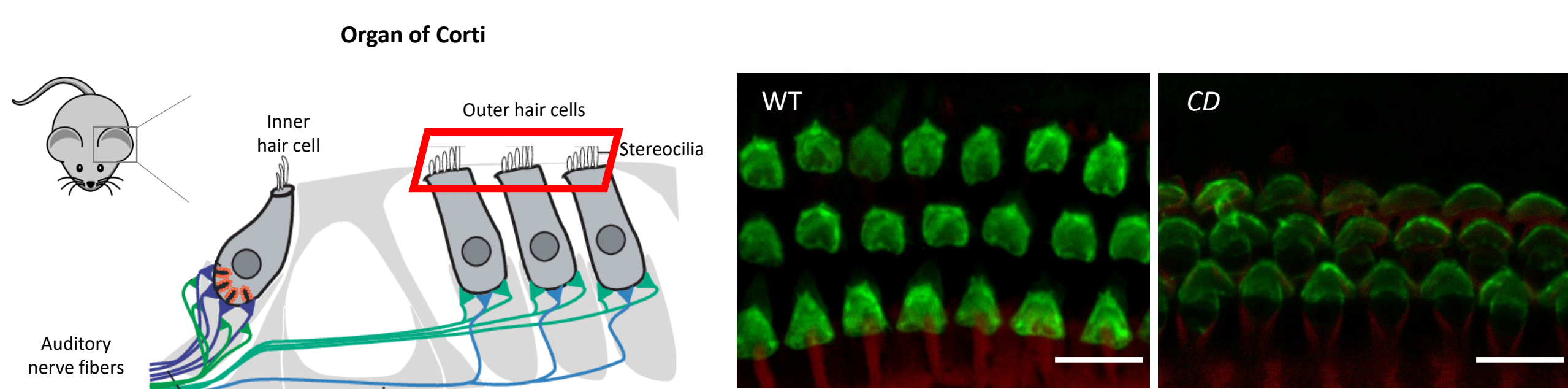
- ❖ To characterize the auditory impairments in *CD* mice, i.e. the mouse model of WBS, carrying out the complete deletion (CD) equivalent to WB patients.
- ❖ To evaluate whether BKCa channels are involved in the auditory impairments of *CD* mice and whether these can be rescued by pharmacological activation through a BKCa opener molecule, i.e., Chlorzoxazone.

## METHODS

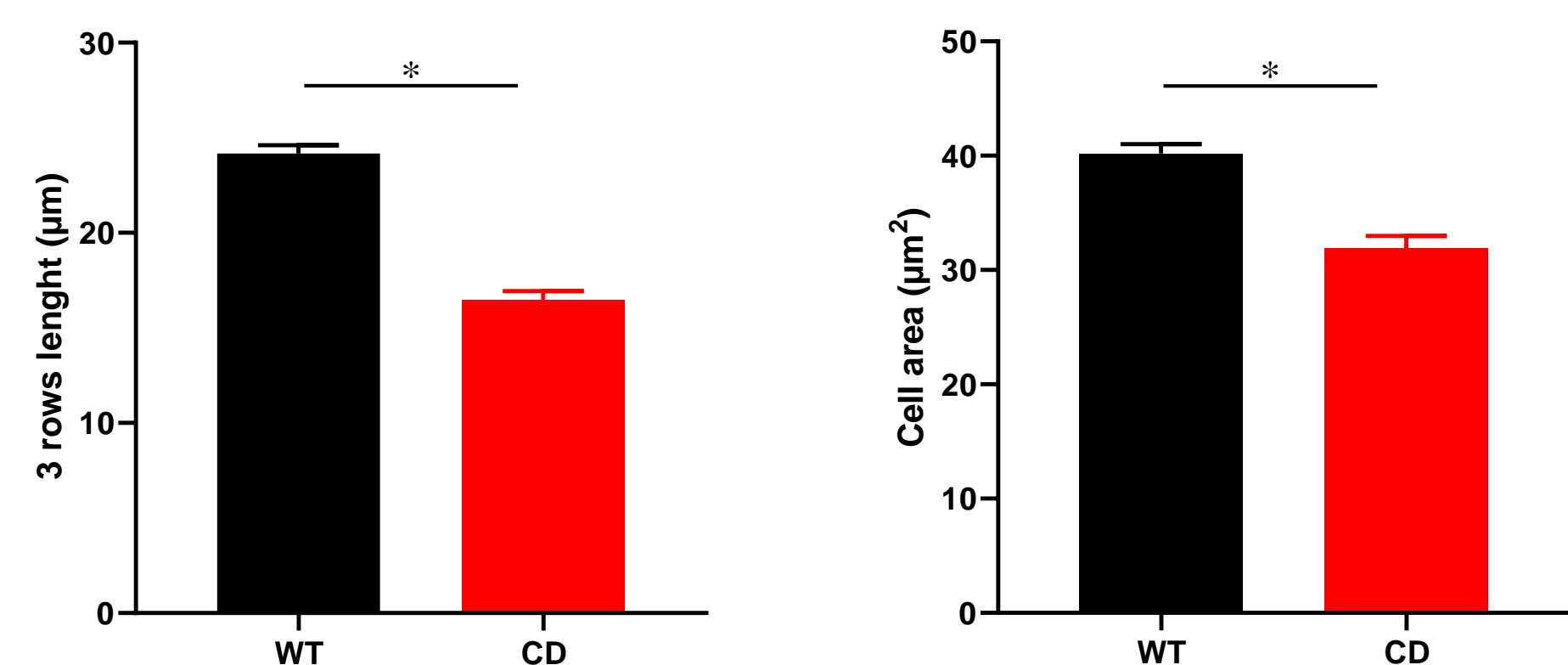
- ❖ Animals: *CD* heterozygous adult males and females B6 (Segura-Puimedon et al. 2014)
- ❖ Pharmacological treatment: acute i.p. injection of a BKCa opener molecule, i.e., Chlorzoxazone (5mg/kg)
- ❖ Immunostaining and confocal microscopy of the organ of Corti and cochlear BKCa channels
- ❖ Electrophysiological test: auditory brainstem response (ABR)
- ❖ Behavioral test: acoustic startle response (ASR)

## IMMUNOSTAINING

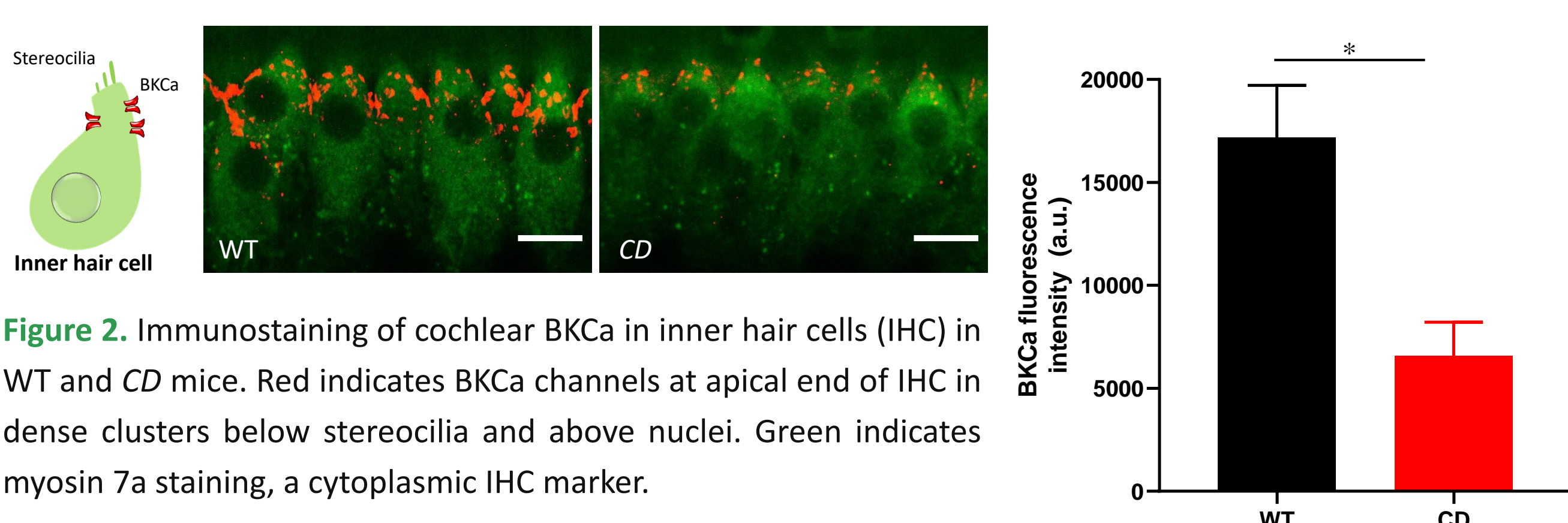
### Quantitative analysis of outer hair cells (OHC)



**Figure 1.** Immunostaining of the outer hair cells (OHC) with focus on stereocilia in WT and *CD* mice. Green indicates oncomodulin staining of three rows of OHC. Red indicates phalloidin staining of the F-actin filament expressed by OHC, inner hair cells (IHC) and a variety of support cells of the organ of Corti.

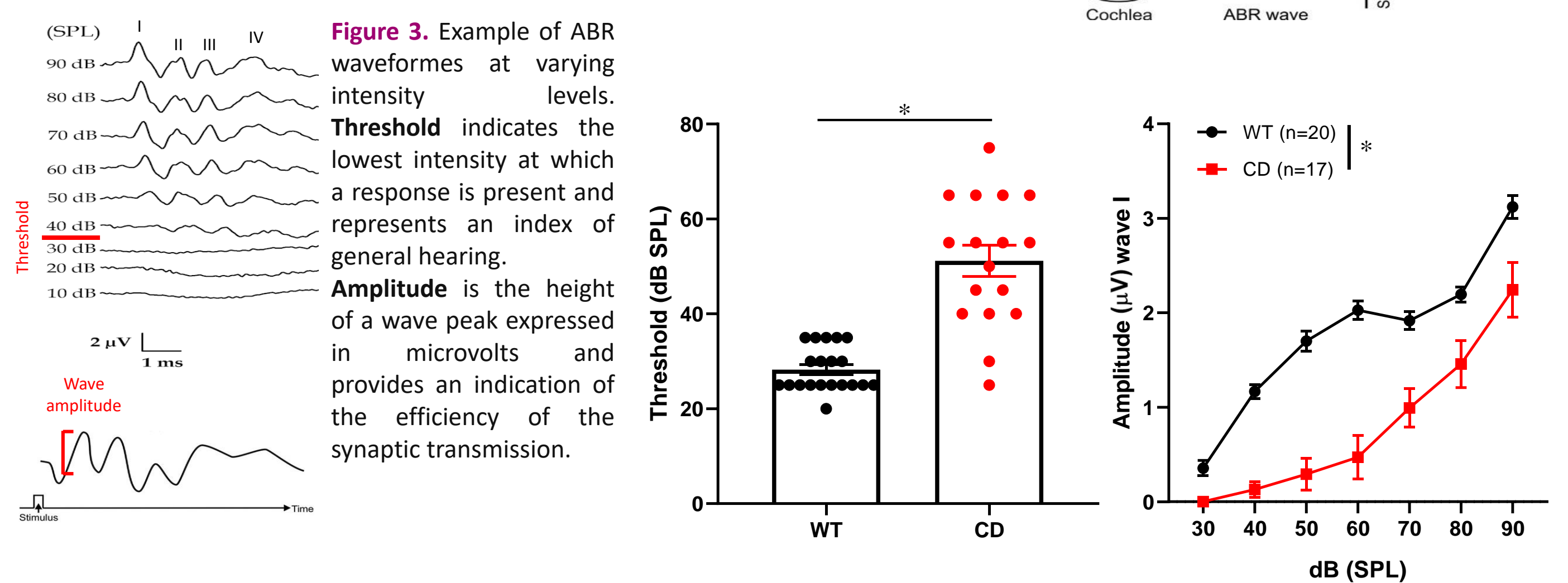
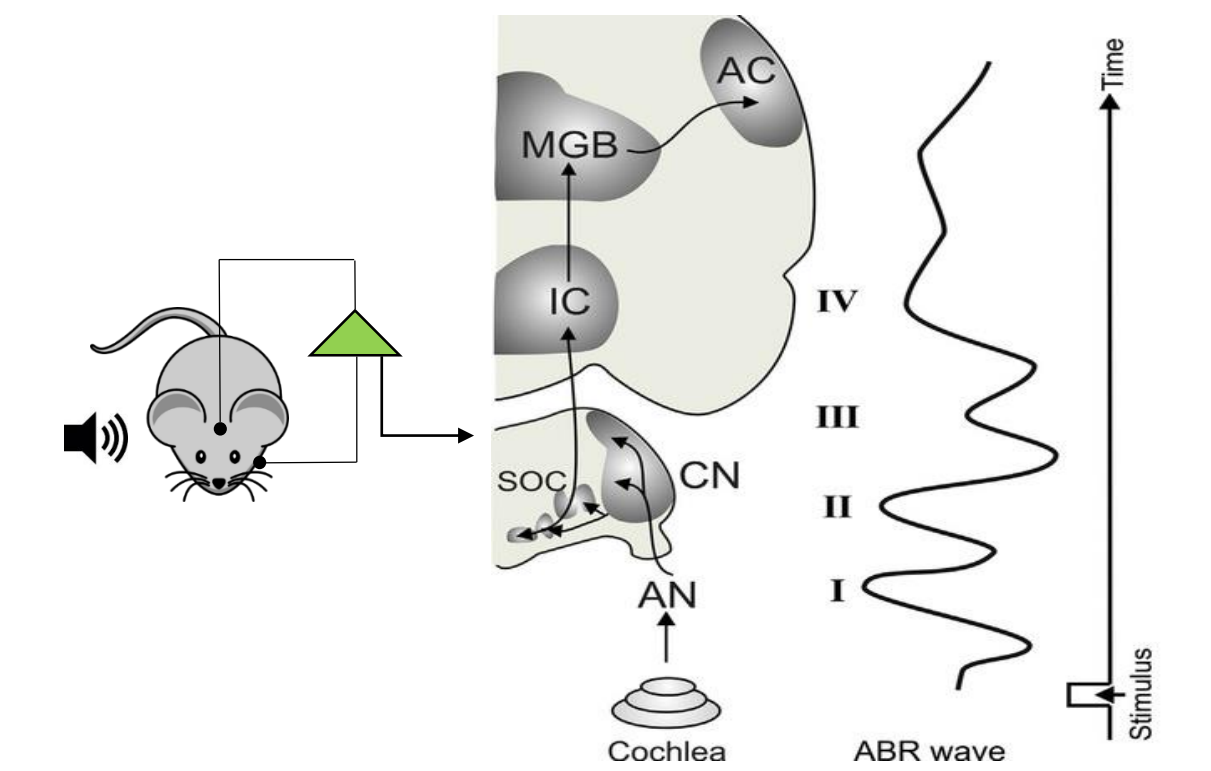
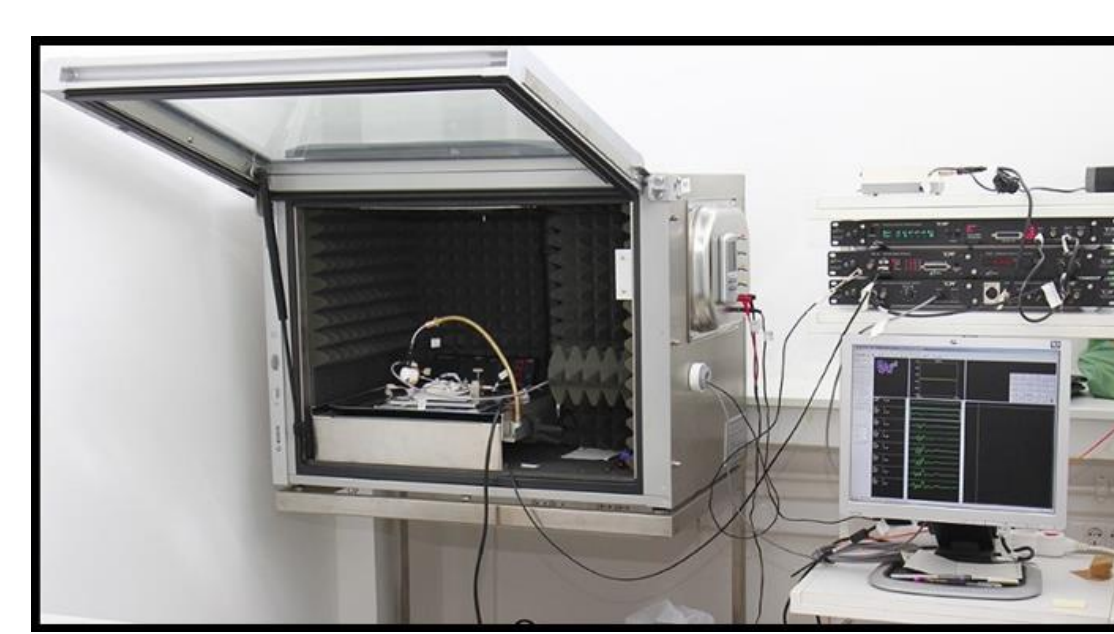


### Quantitative analysis of cochlear BKCa channels



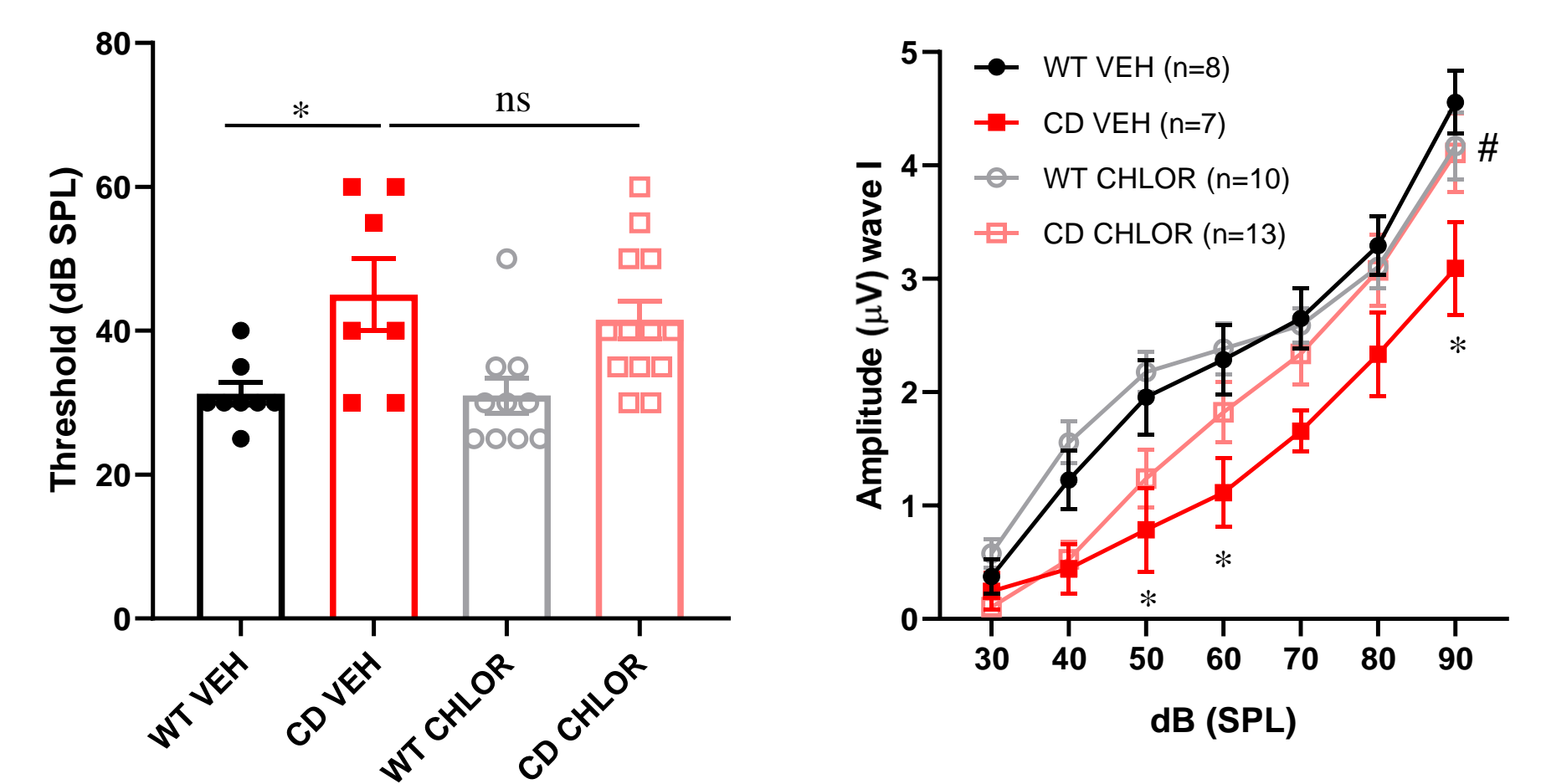
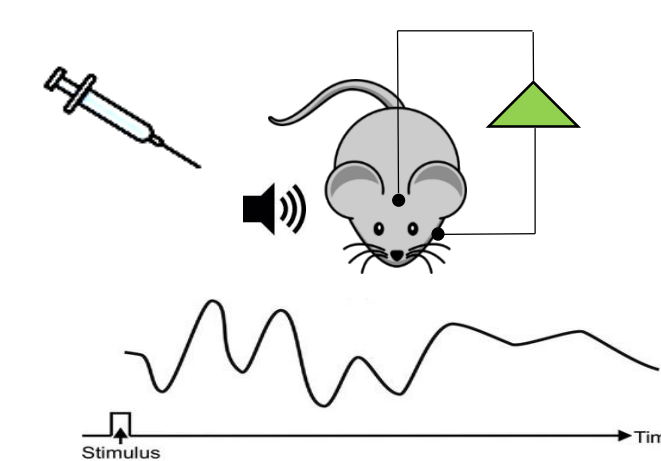
**Figure 2.** Immunostaining of cochlear BKCa in inner hair cells (IHC) in WT and *CD* mice. Red indicates BKCa channels at apical end of IHC in dense clusters below stereocilia and above nuclei. Green indicates myosin 7a staining, a cytoplasmic IHC marker.

## AUDITORY BRAINSTEM RESPONSE



## PHARMACOLOGICAL TREATMENT

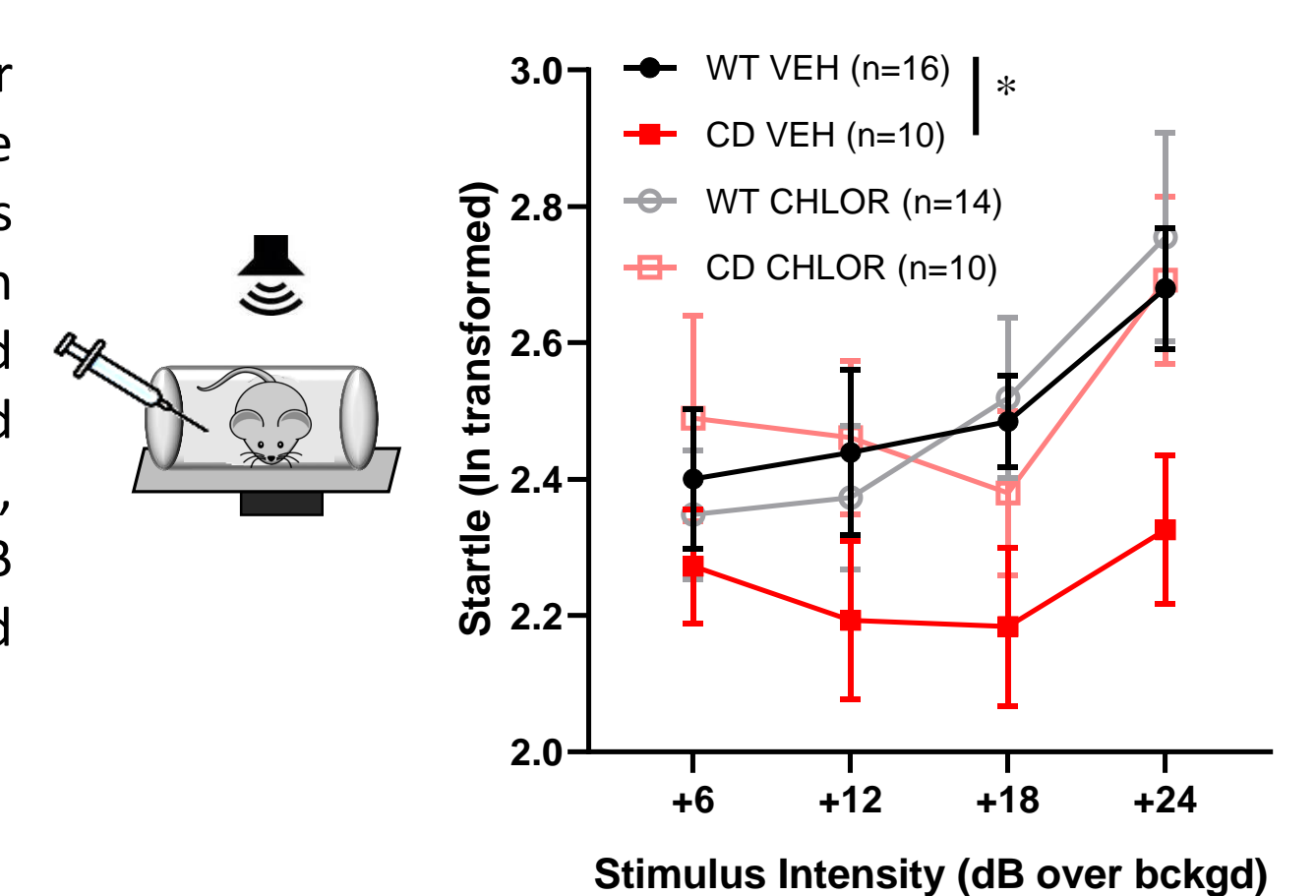
### Auditory brainstem response + Chlorzoxazone



### Acoustic startle response + Chlorzoxazone



**Figure 4.** Apparatus for measurement of the ASR in mice. Animals were presented with pulses of white sound of 20 ms duration and varying intensity: +6, +12 +18 and +24 dB over the 66dB bckgd level.



## CONCLUSIONS

- ❖ Cochlear immunostaining in *CD* mice revealed:
  - Morphological abnormalities of OHC stereocilia, which are responsible for amplifying the incoming sound.
  - Reduced expression of BKCa channels in IHC, which strongly contribute to the synaptic transmission from the cochlea to the auditory nerve.
- ❖ ABR recordings revealed increased threshold and decreased amplitude in *CD* mice, which together indicate significant hearing loss due to a reduced efficiency of the synaptic transmission.
- ❖ Acute Chlorzoxazone injection tends to increase ABR amplitude in *CD* mice, especially at the highest intensities (significant at 90dB). Nonetheless, it is not sufficient to rescue their increased threshold.
- ❖ *CD* mice display reduced ASR, which is consistent with their increased threshold and the hearing loss described in WB patients. Acute Chlorzoxazone injection ameliorates their ASR performance.