

DNA Repair Interest Group Seminar

Short-term NAD⁺ supplementation prevents hearing loss in mouse models of Cockayne syndrome

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National Institute on Aging

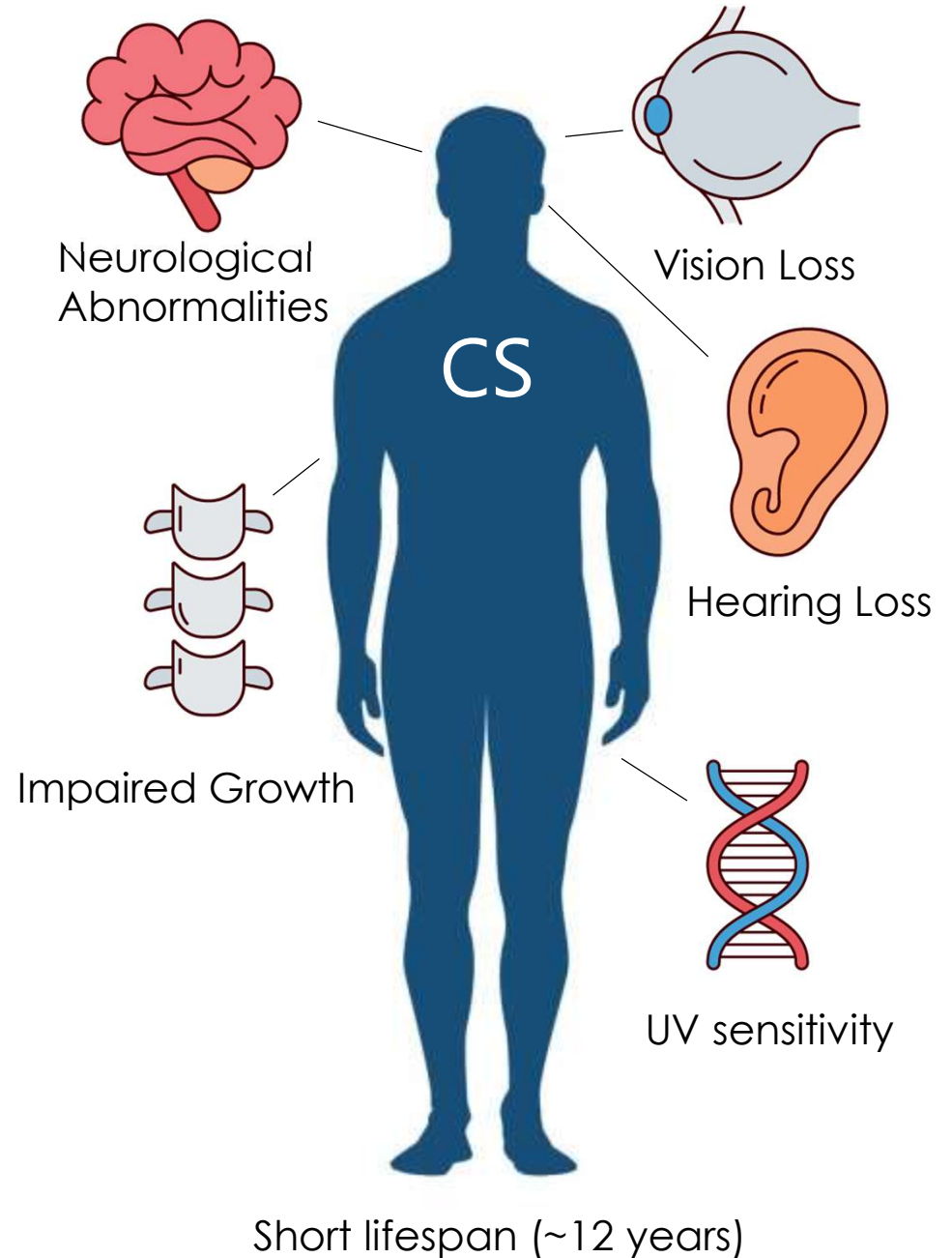


Cockayne syndrome (CS) is a segmental premature aging genetic disease



Wilson et al., 2015

CS is an autosomal recessive genetic disorder, due to mutations predominantly in **CSA** and **CSB** coding genes.



CSA and CSB structures



protein



WD40 motifs
(7 repeats)

A subunit of
CUL4A-DDB1 E-LIGASE
complex.



protein

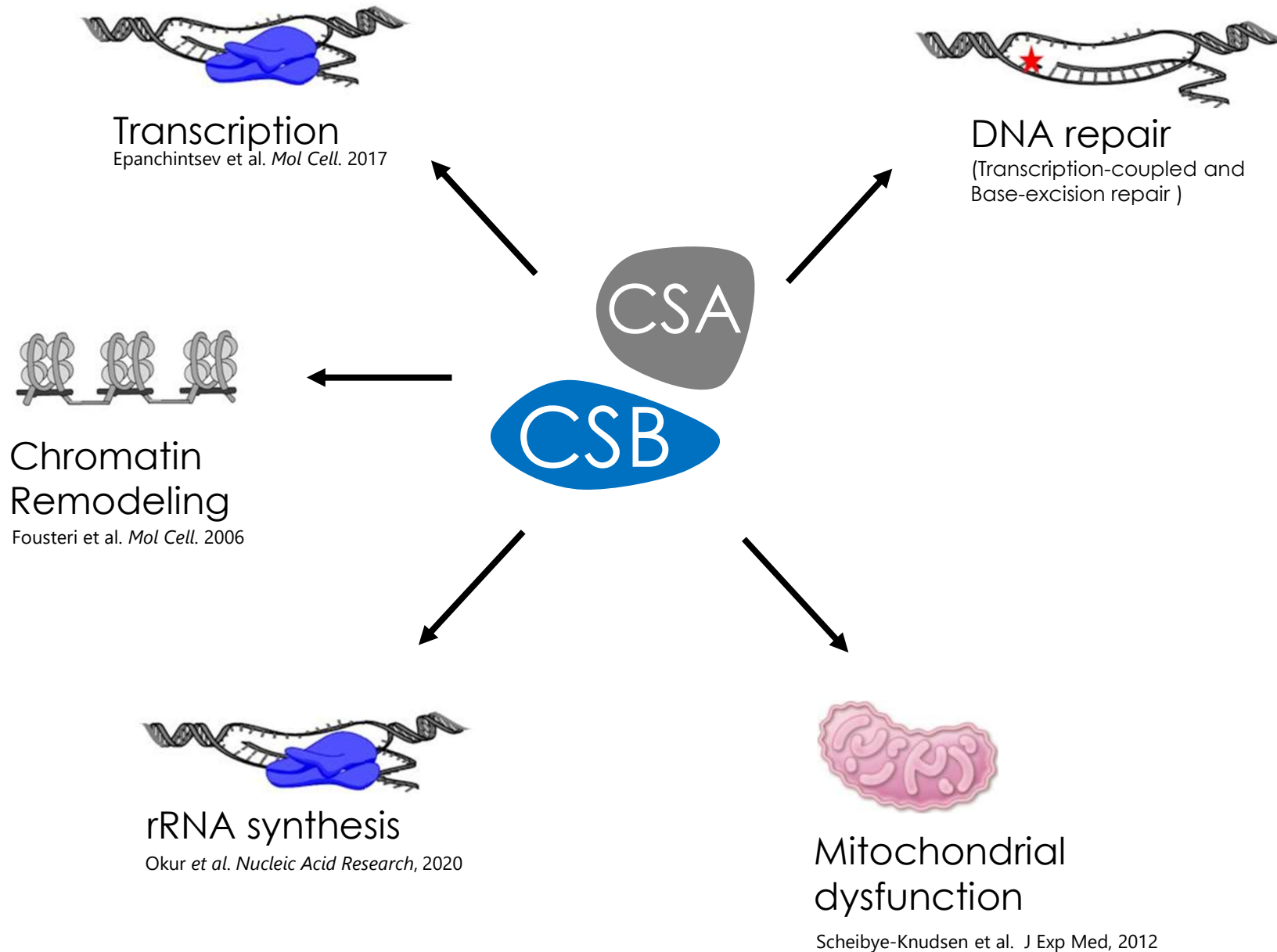


ATPase Domain

UBD Domain

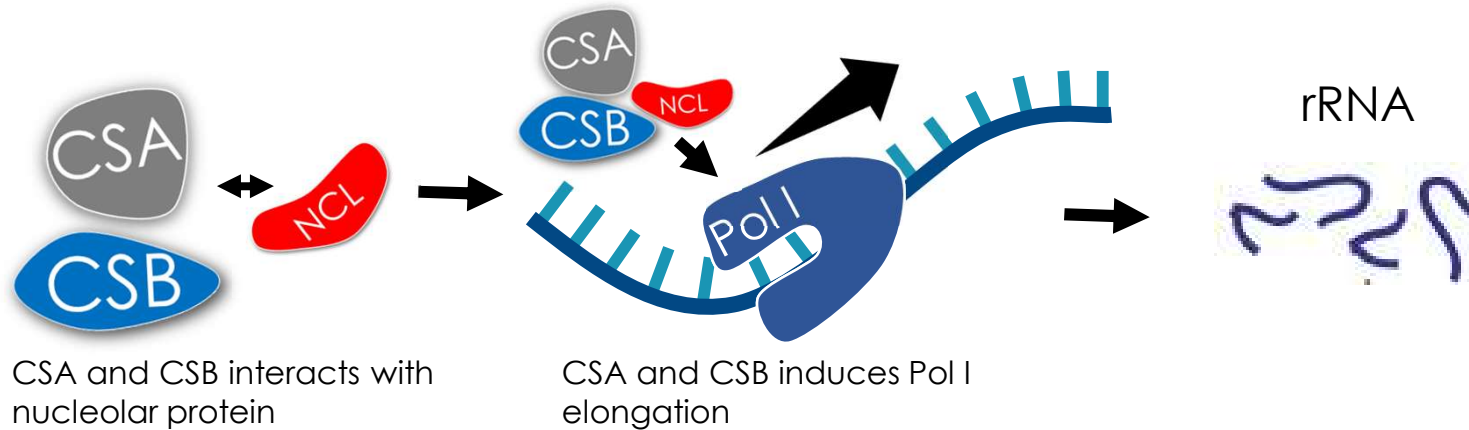
CSB has DNA-dependent ATPase and Ubiquitin
Binding Domain (UBD)

CSA and CSB are linked to multiple biological functions

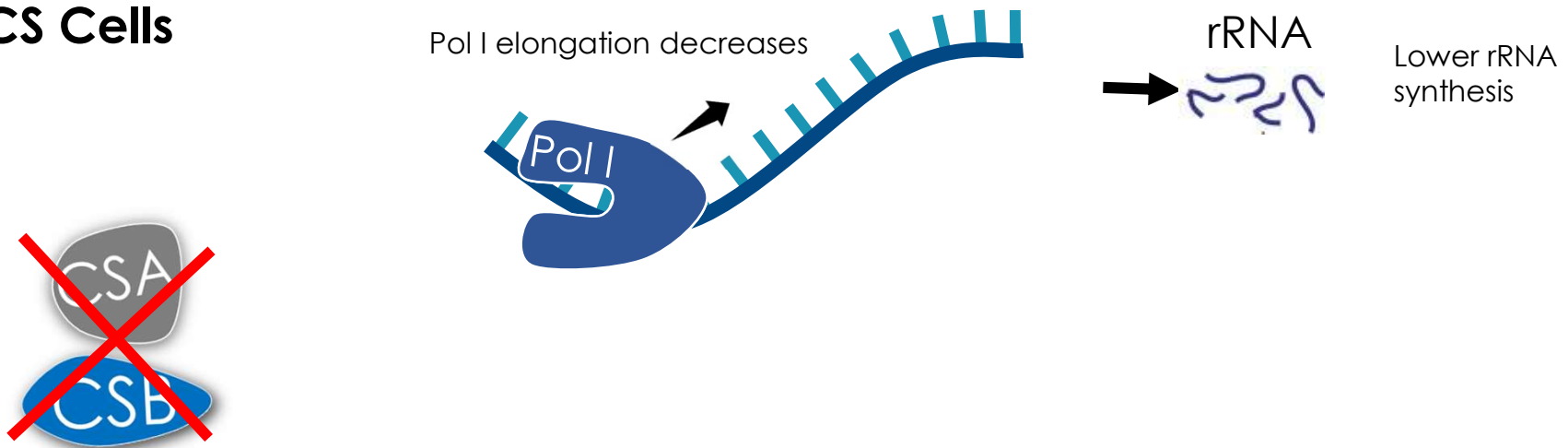


Convergence of CSA and CSB at rRNA synthesis

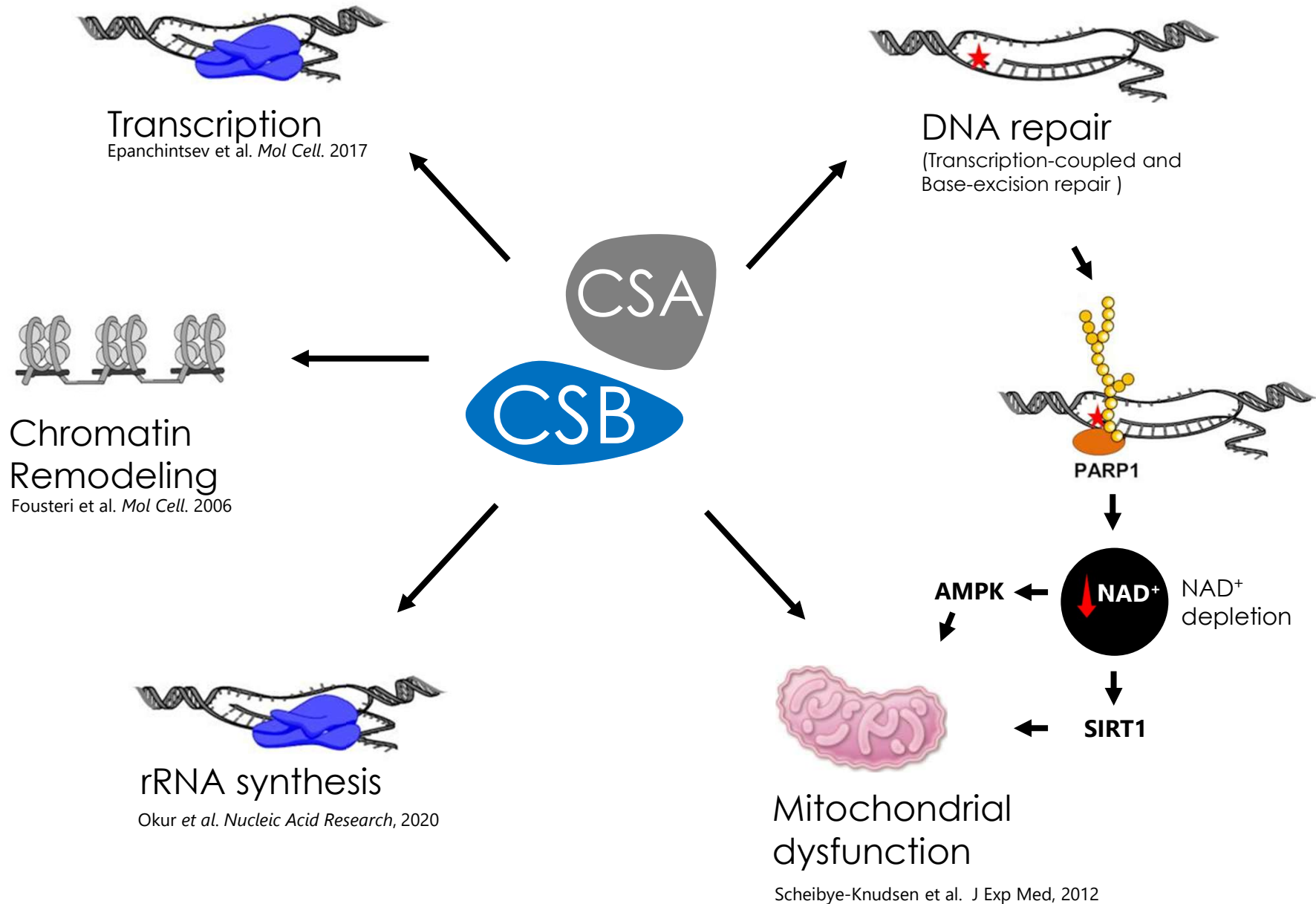
Healthy Cells



CS Cells



CSA and CSB are linked to multiple biological functions

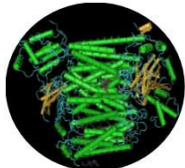


Nicotinamide adenine dinucleotide (**NAD⁺**)

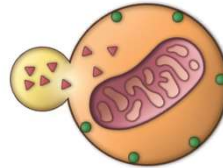
NAD⁺ a key metabolite that functions as a cofactor to activate multiple enzymes



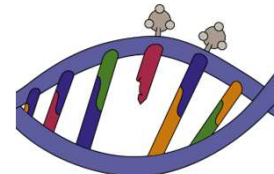
Mitochondrial dysfunction



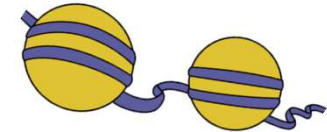
Loss of proteostasis



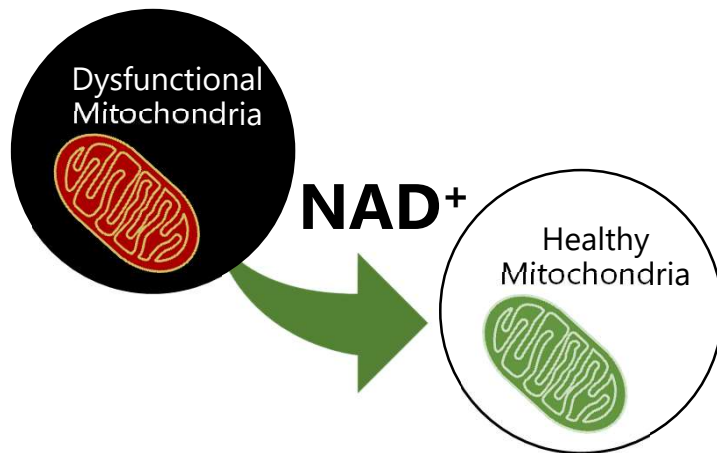
Compromised autophagy



DNA damage



Epigenetic alterations

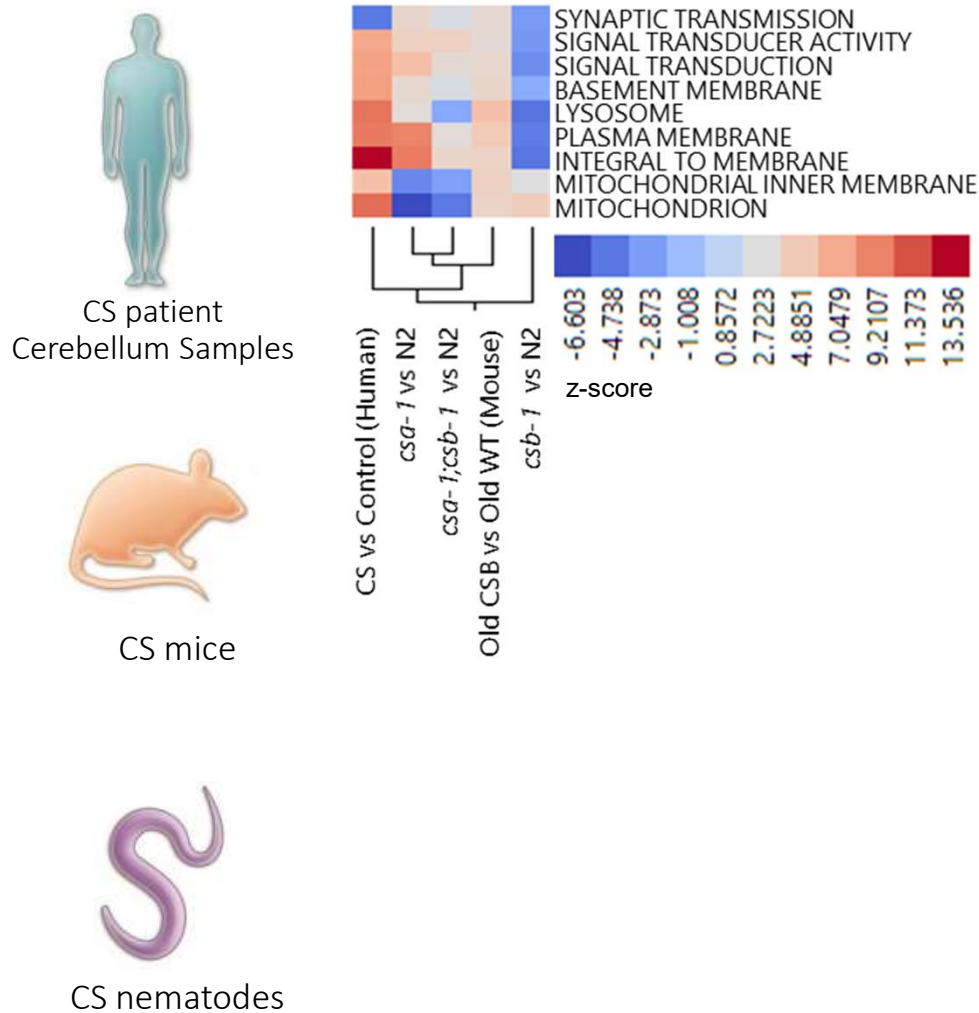


NAD⁺ declines with age in mice.

Treatment with exogenous NAD⁺ improves mitochondrial function and increases life span in mice.

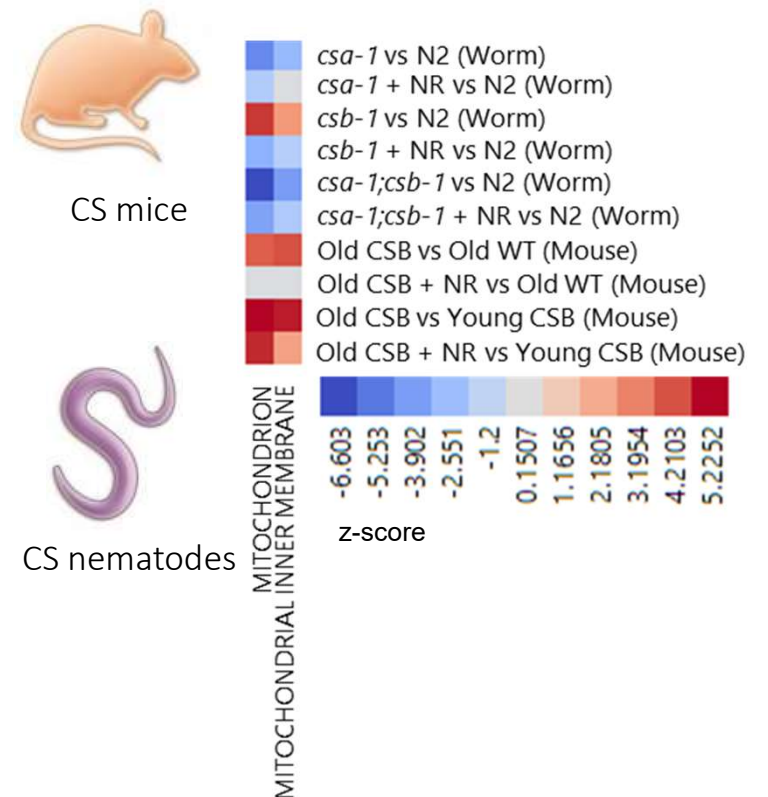
Fang *et al. Cell Metab.*, 2016
Schultz *et al. Cell Metab.*, 2016
Zhang *et al. Science*, 2016

Transcriptomic Analysis on CS models and NAD⁺ supplementation

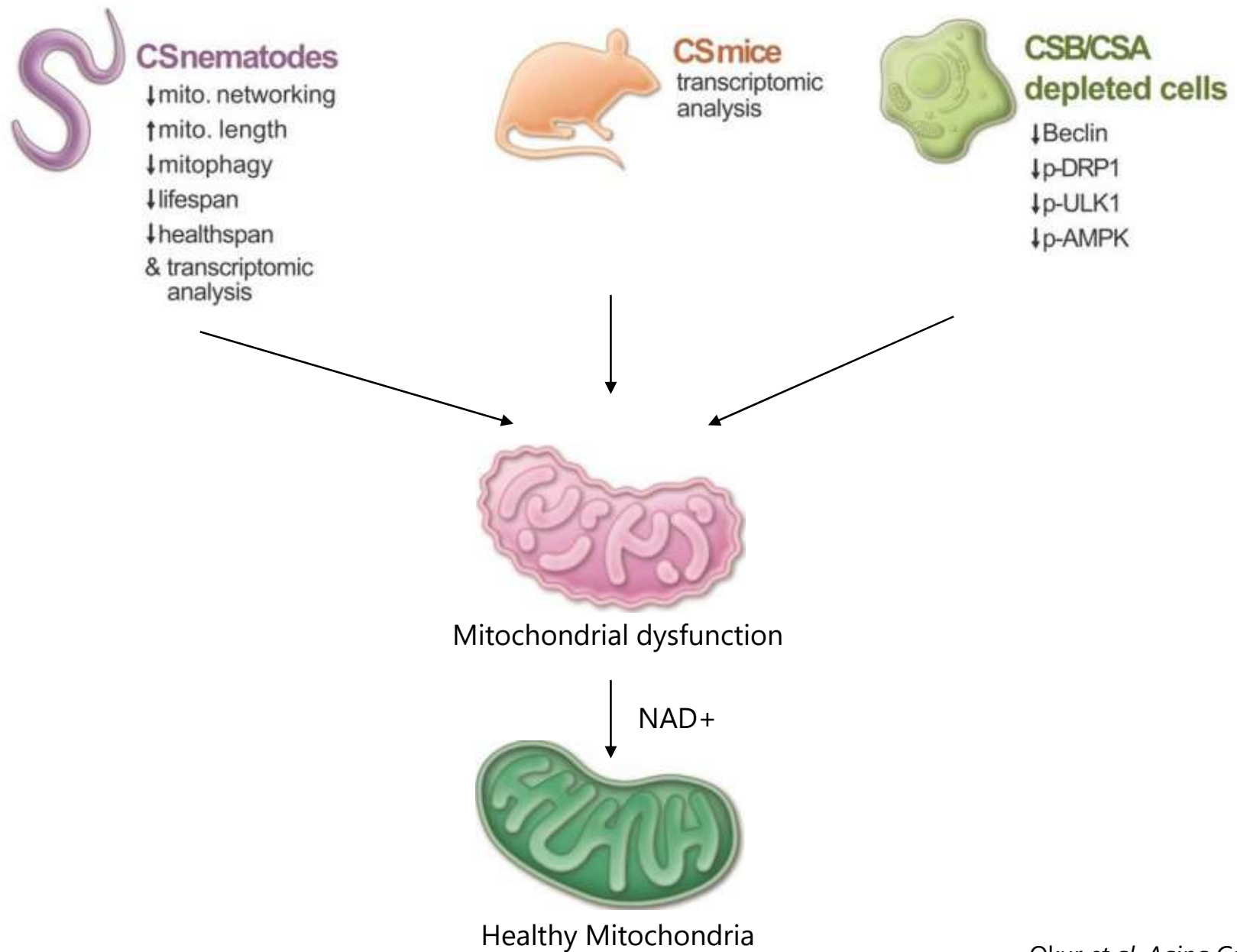


Mitochondrial terms in CS models are restored with NAD⁺ supplementation

Nicotinamide Riboside is used as NAD⁺ precursor



Mitochondrial dysfunction in CS models is restored with NAD⁺ supplementation



Mitochondrial dysfunction plays a casual role in hearing loss

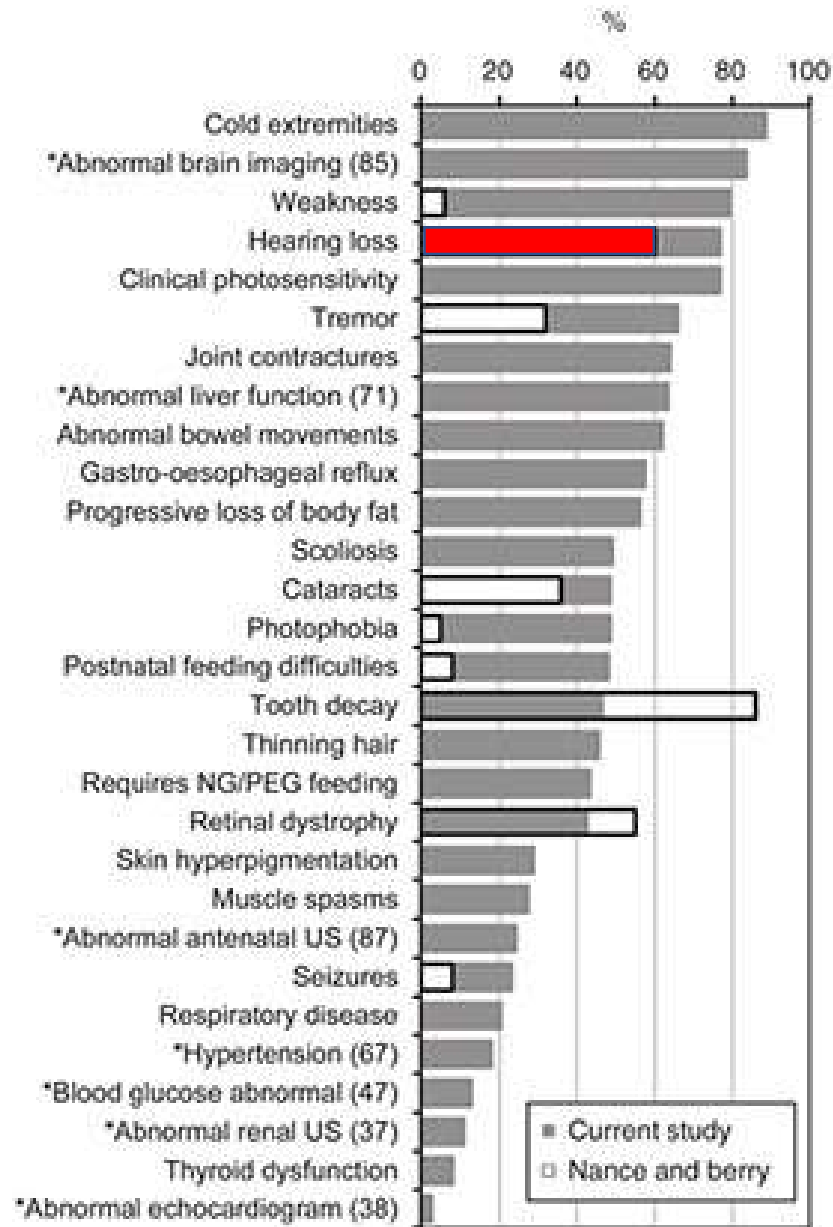


The decline of mitochondrial function is one of the major theories proposed for the development of hearing loss.

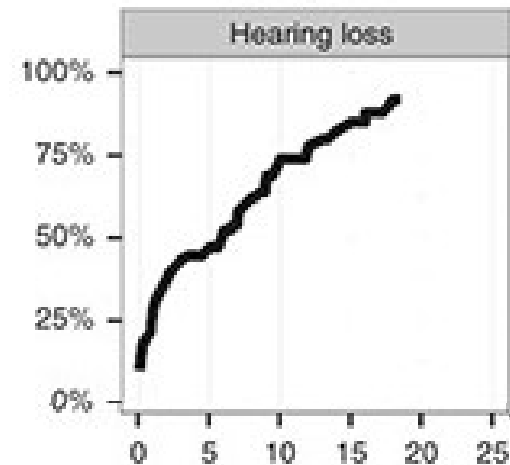
Hearing impairments are often seen in patients with mitochondrial diseases.

Fischel-Ghodsian, N et al. *Mitochondrion* 2004.

Hearing loss is a cardinal clinical feature of CS

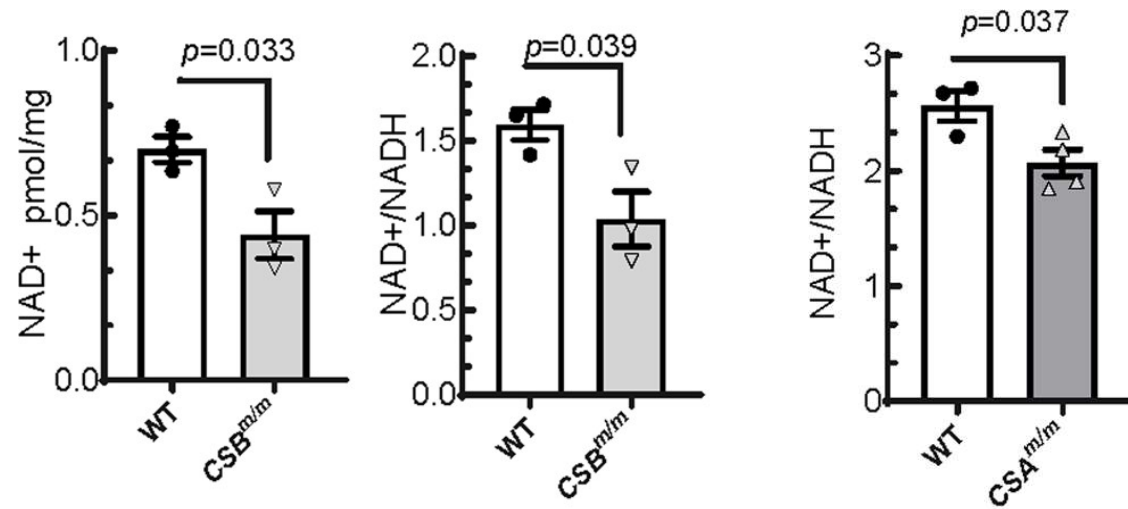


~75% of CS patients by the age of 10

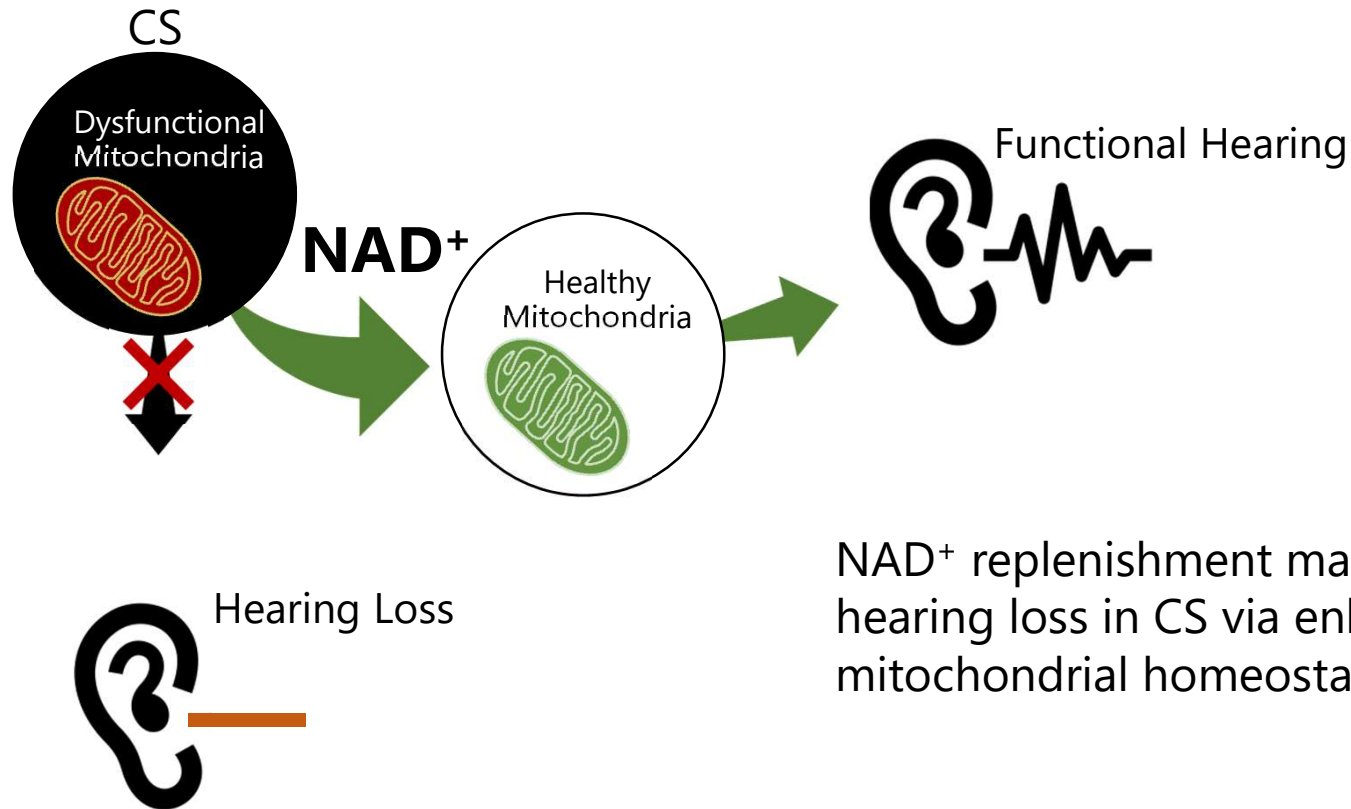


Hearing loss is recapitulated in the mouse models of CS

NAD⁺ levels are lower in CS cochlea

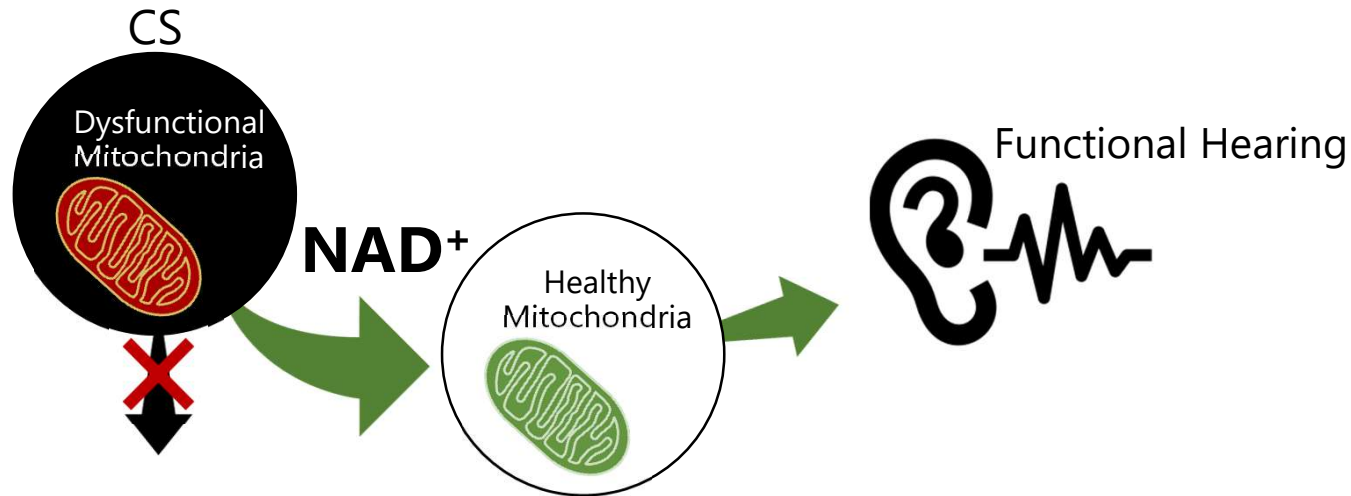


Hypothesis/Research Plan

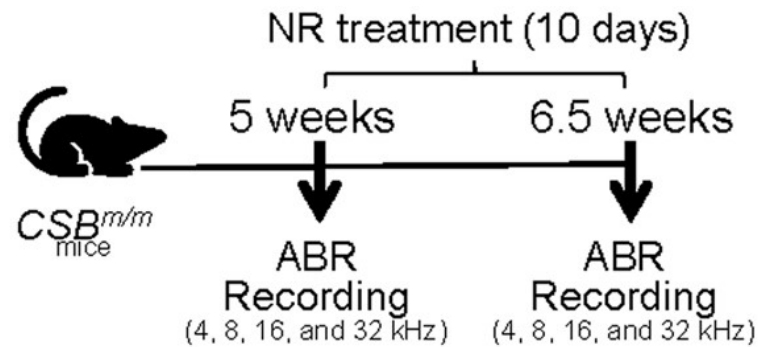


NAD^+ replenishment may ameliorate hearing loss in CS via enhancing mitochondrial homeostasis.

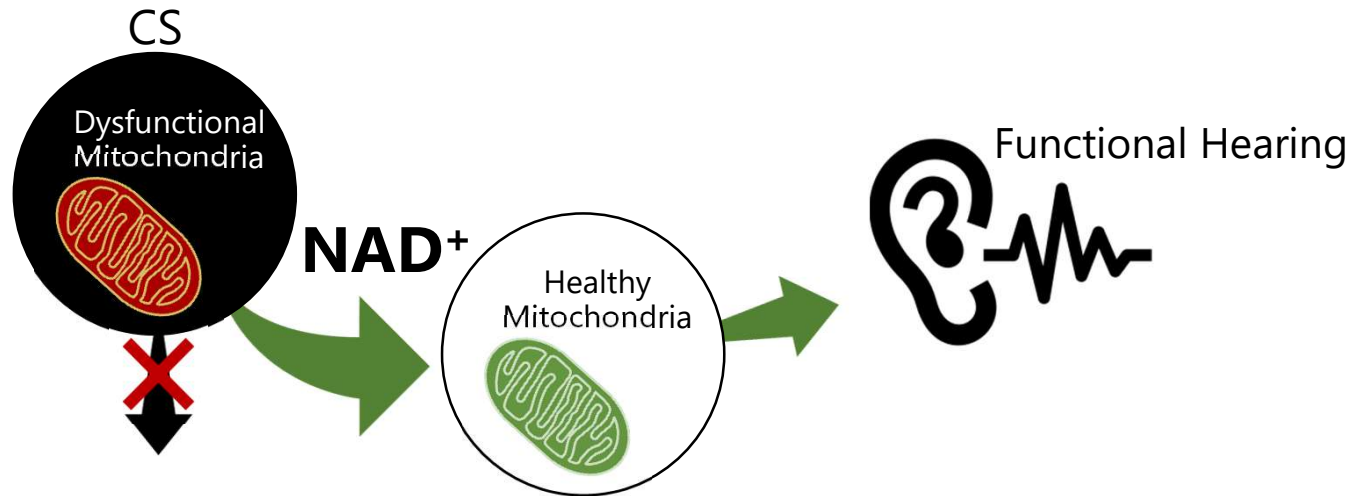
Hypothesis/Research Plan



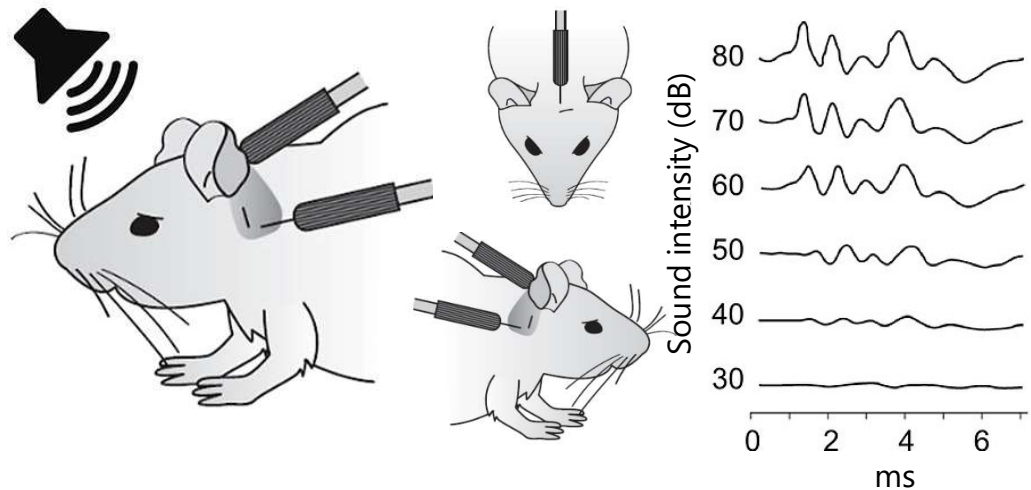
Nicotinamide Riboside (NR) is used as NAD⁺ precursor.



Hypothesis/Research Plan

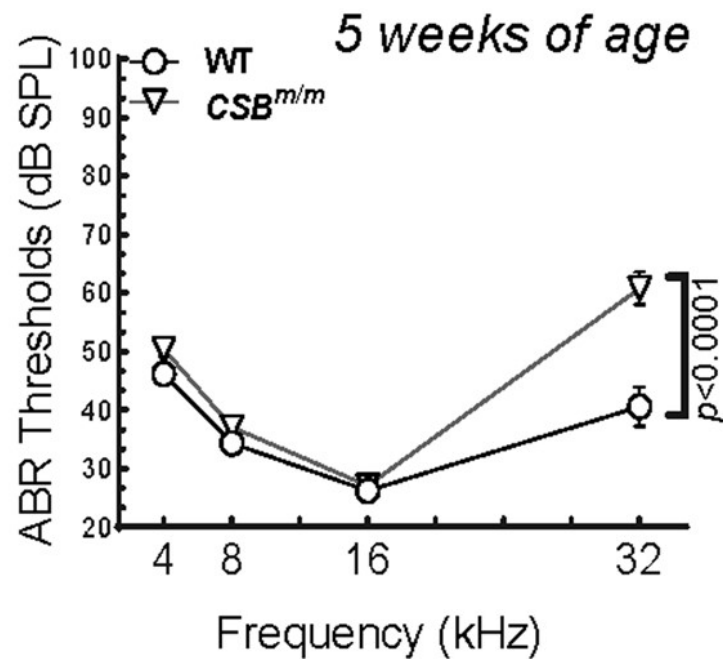


Auditory Brainstem Response (ABR) is used to measure hearing capacity in mice

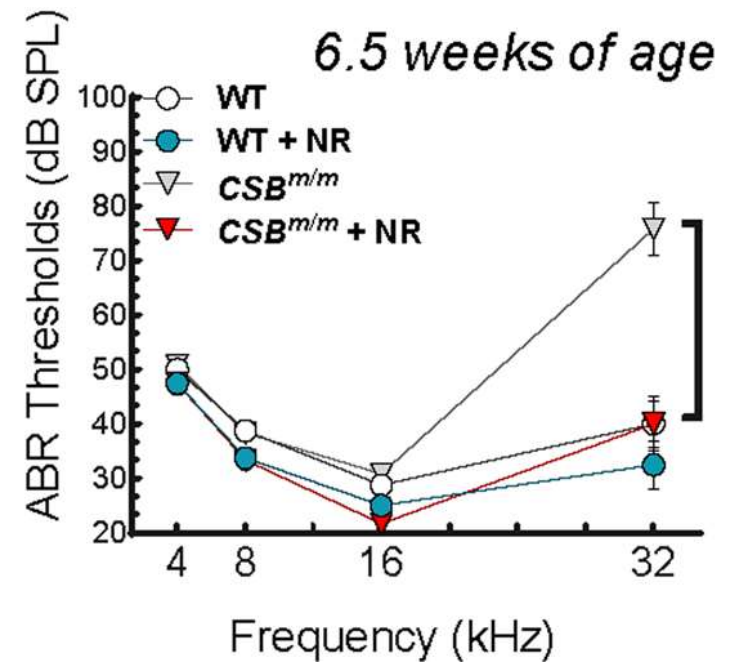


Brain wave activity over time (ms)

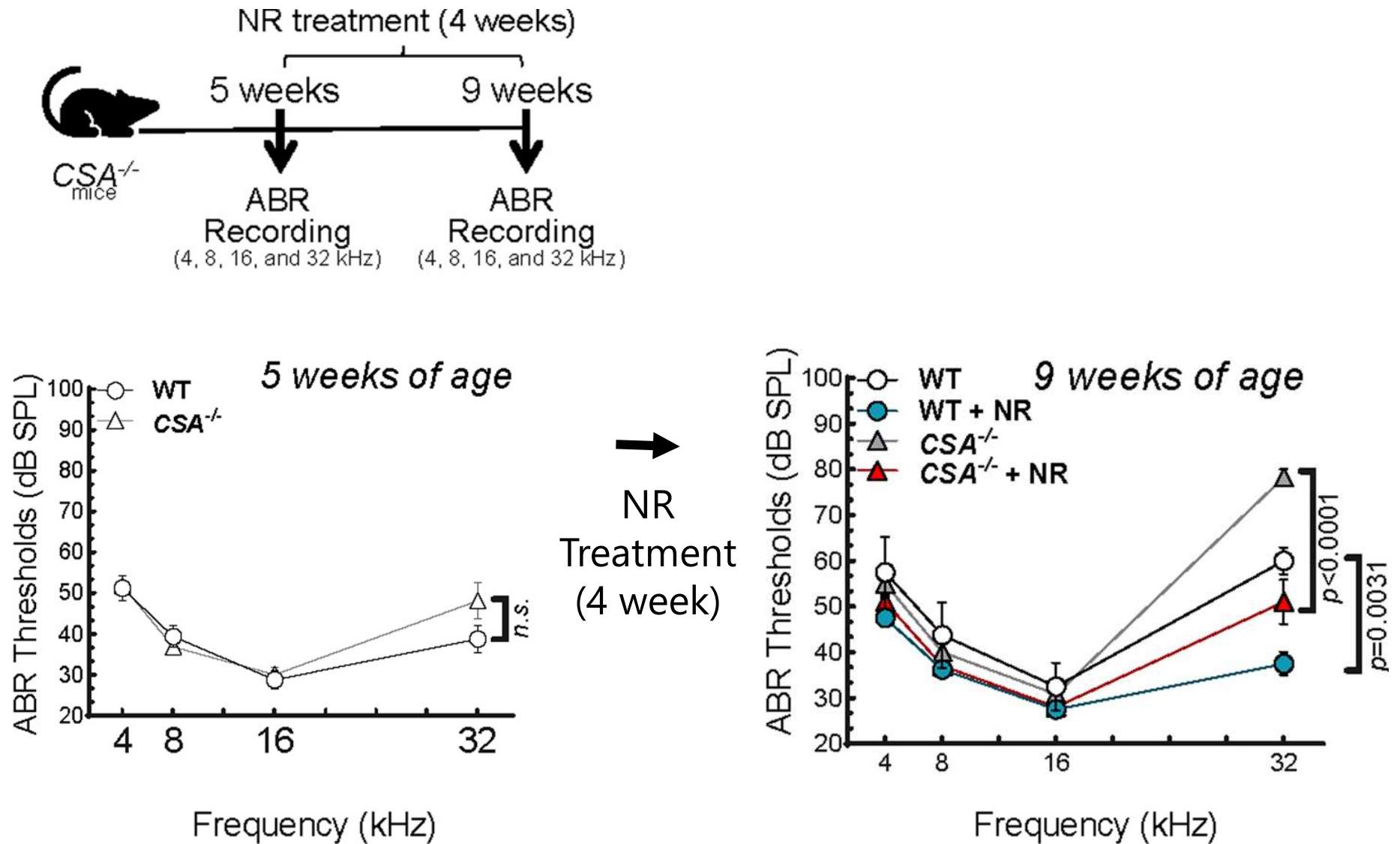
NR treatment prevents the progression of hearing loss in $CSB^{m/m}$ mice



NR
Treatment
(10 days)

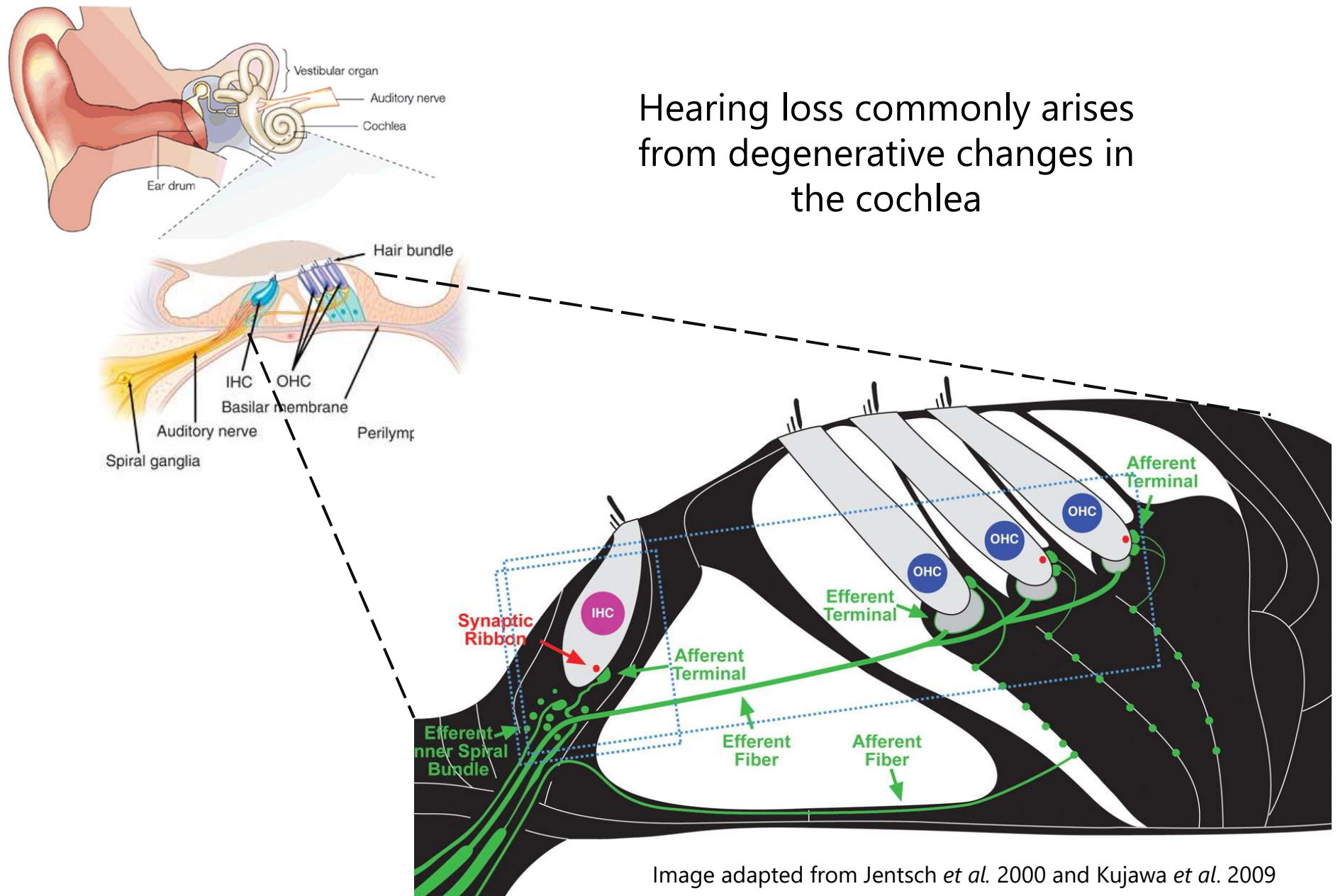


NR treatment prevents the progression of hearing loss in $CSA^{-/-}$ mice



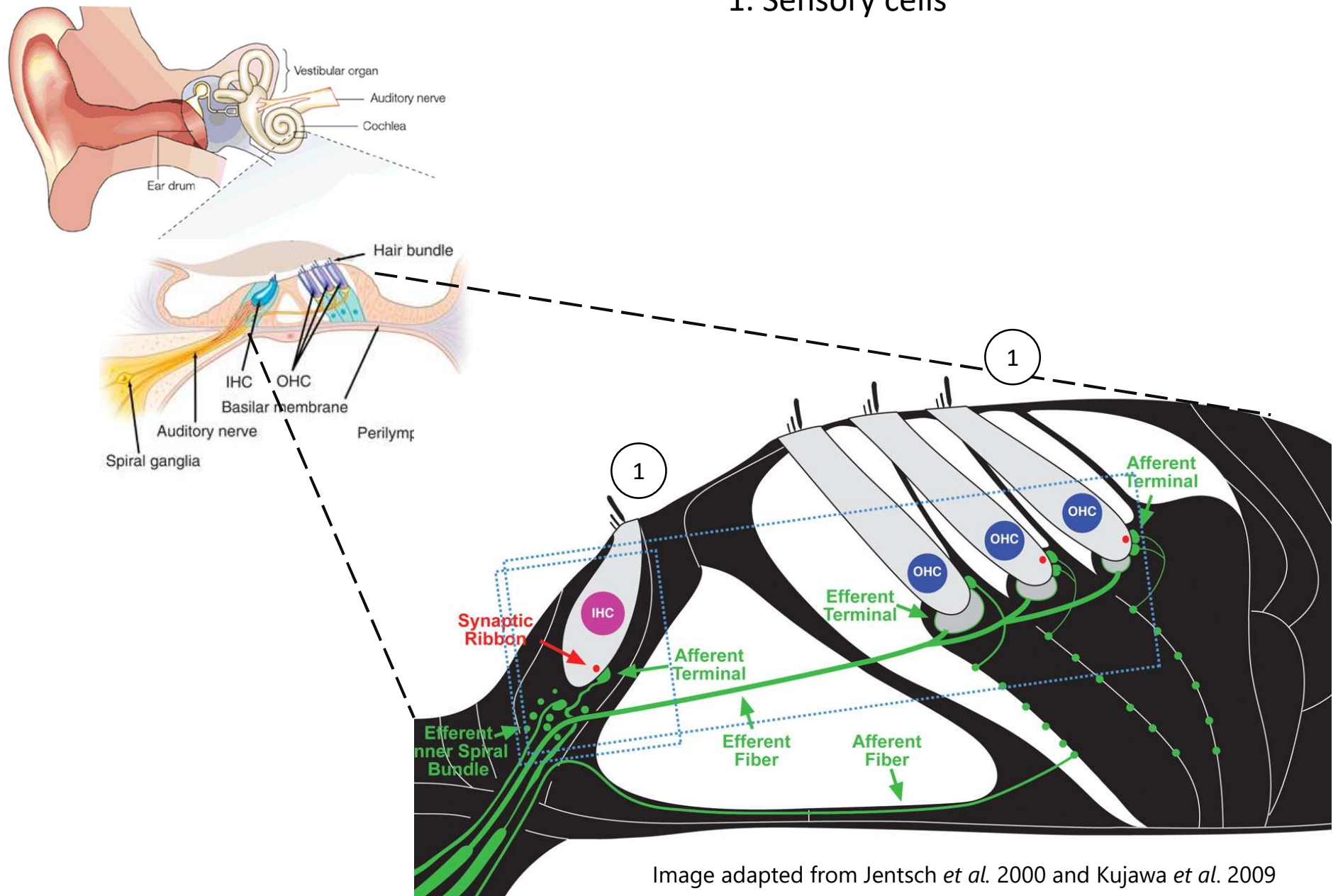
Potential mechanisms of protective effect of NR treatment on hearing in CS mice?

Hearing loss commonly arises from degenerative changes in the cochlea



Hearing loss commonly arises from degenerative changes in the cochlea

1. Sensory cells



Hearing loss commonly arises from degenerative changes in the cochlea

1. Sensory cells
2. Spiral ganglion neurons

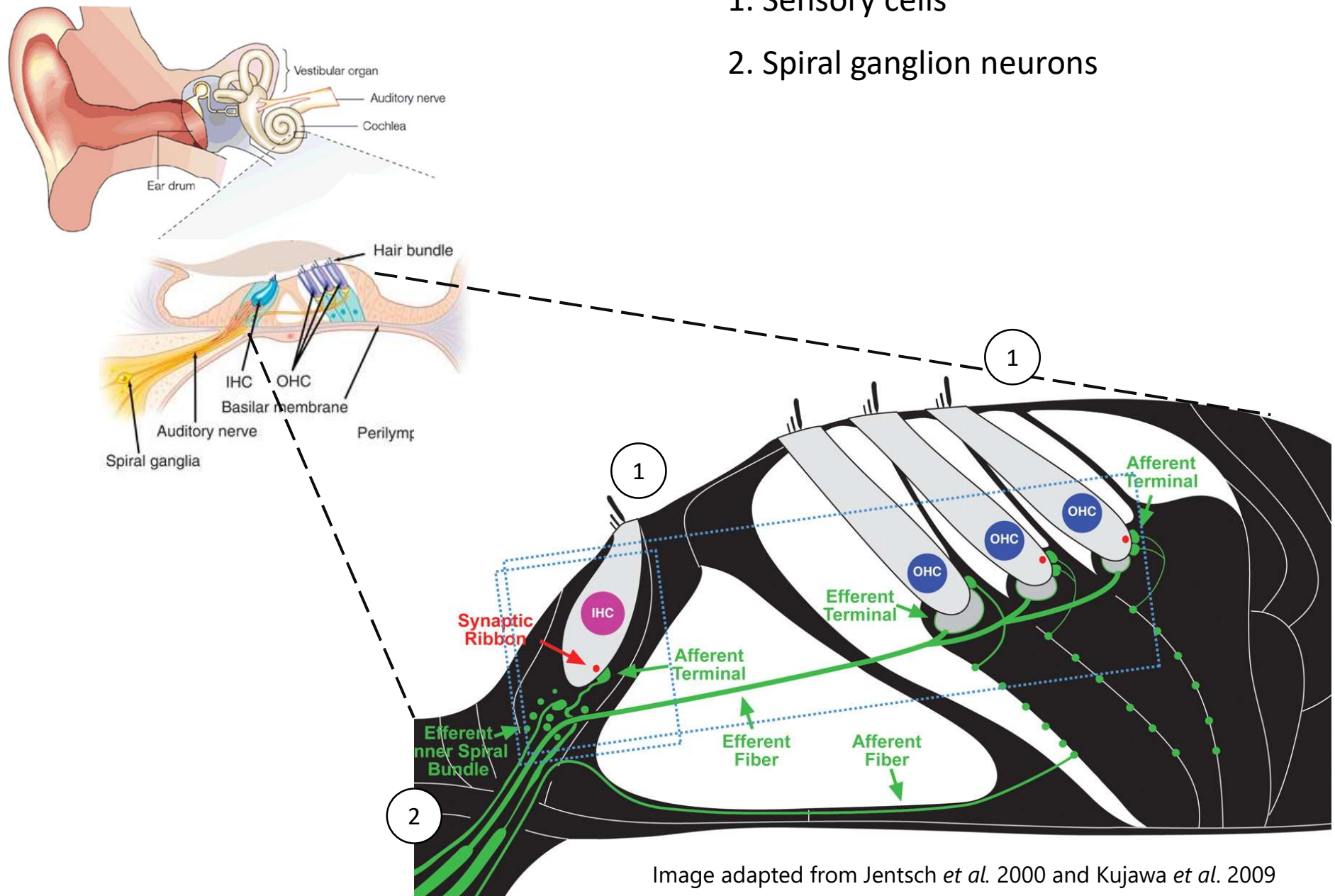
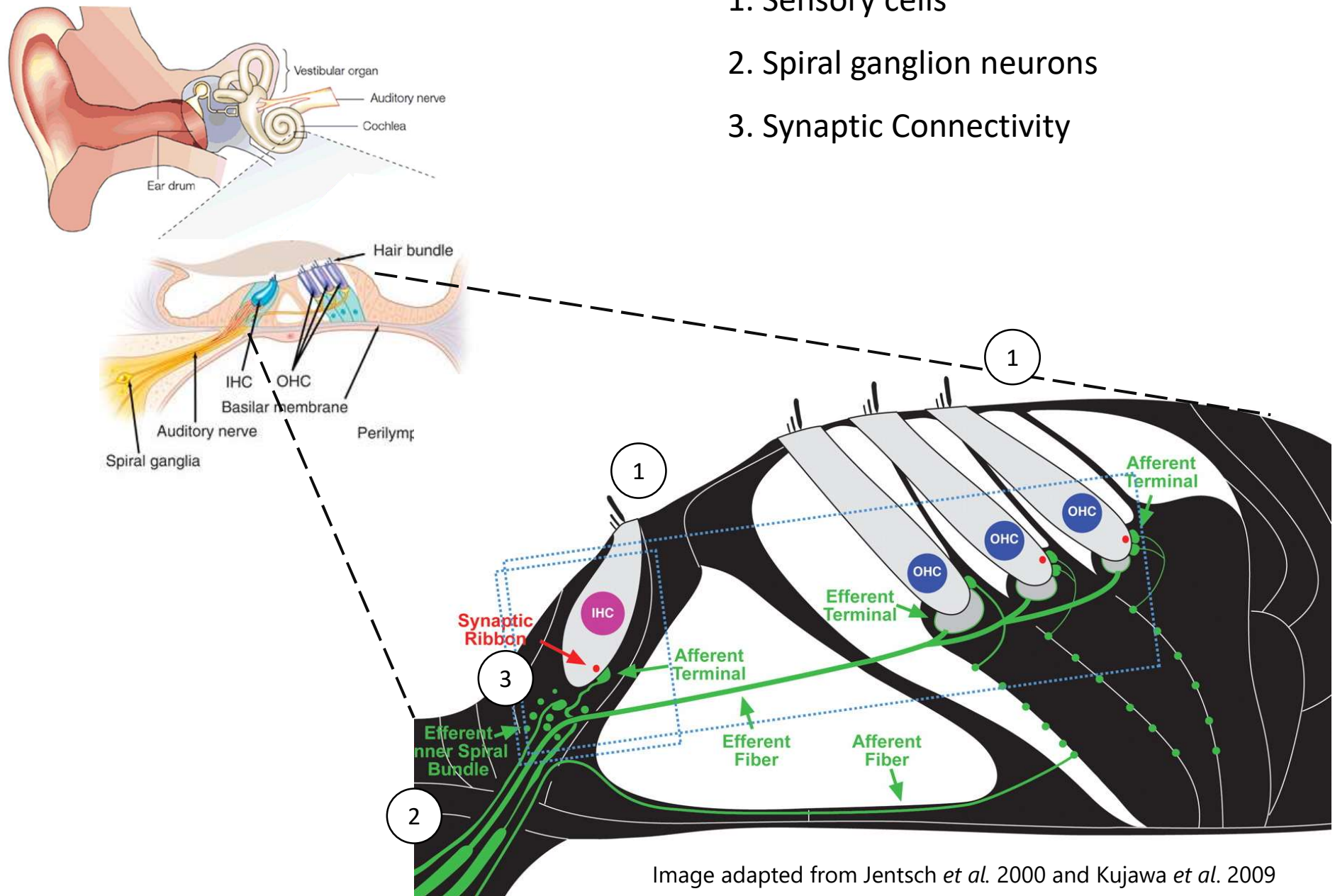


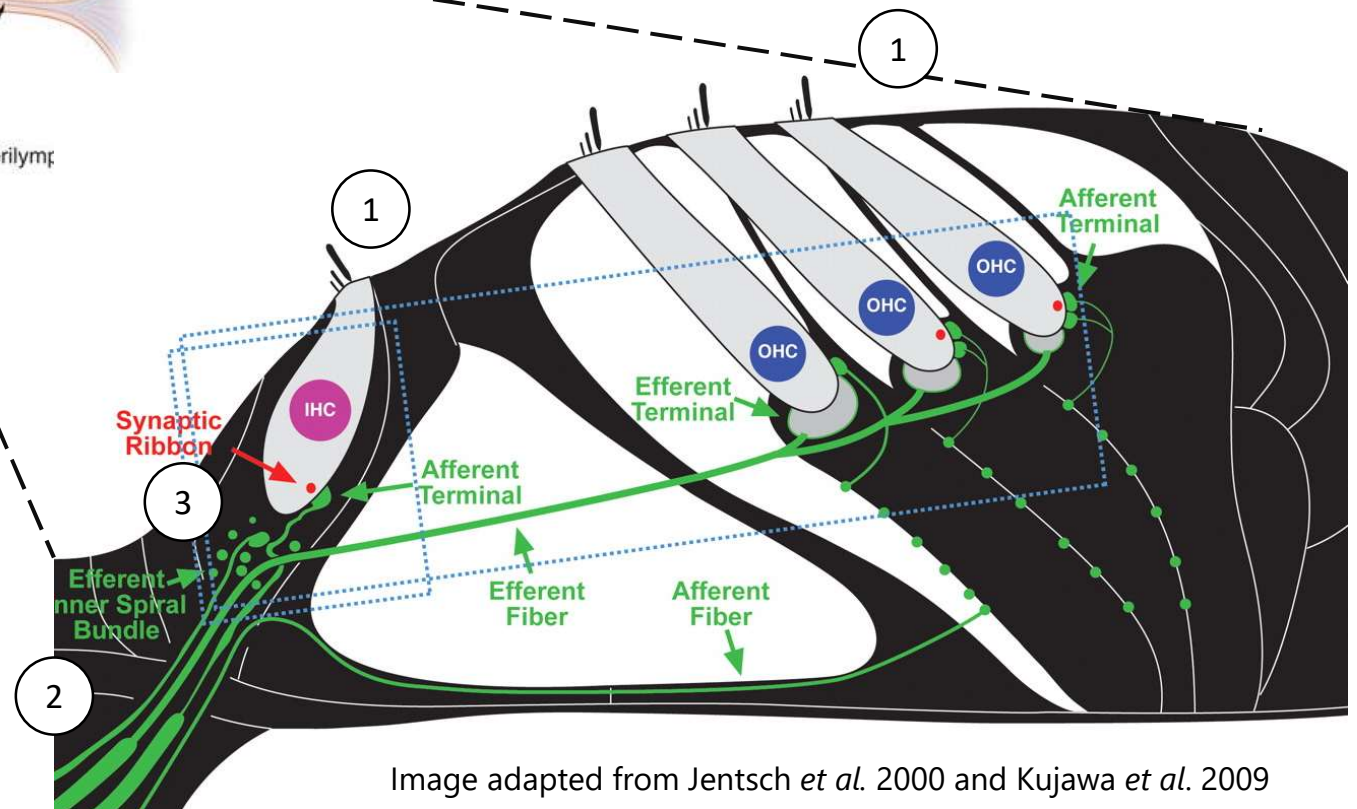
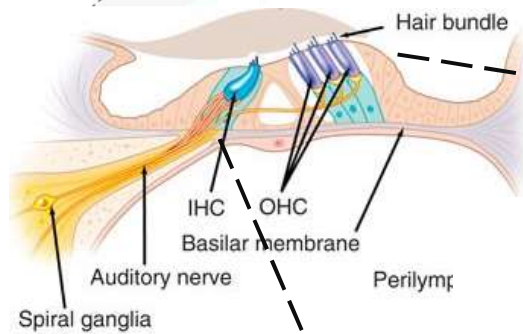
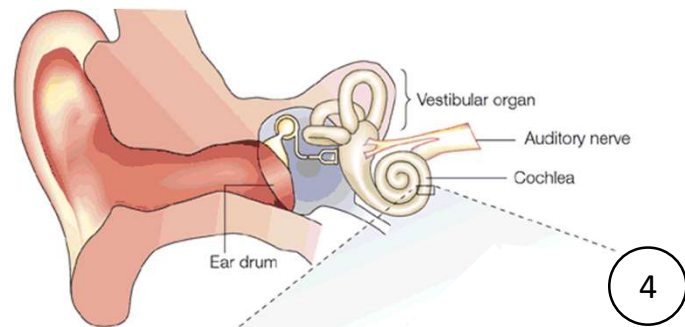
Image adapted from Jentsch *et al.* 2000 and Kujawa *et al.* 2009

Hearing loss commonly arises from degenerative changes in the cochlea

1. Sensory cells
2. Spiral ganglion neurons
3. Synaptic Connectivity



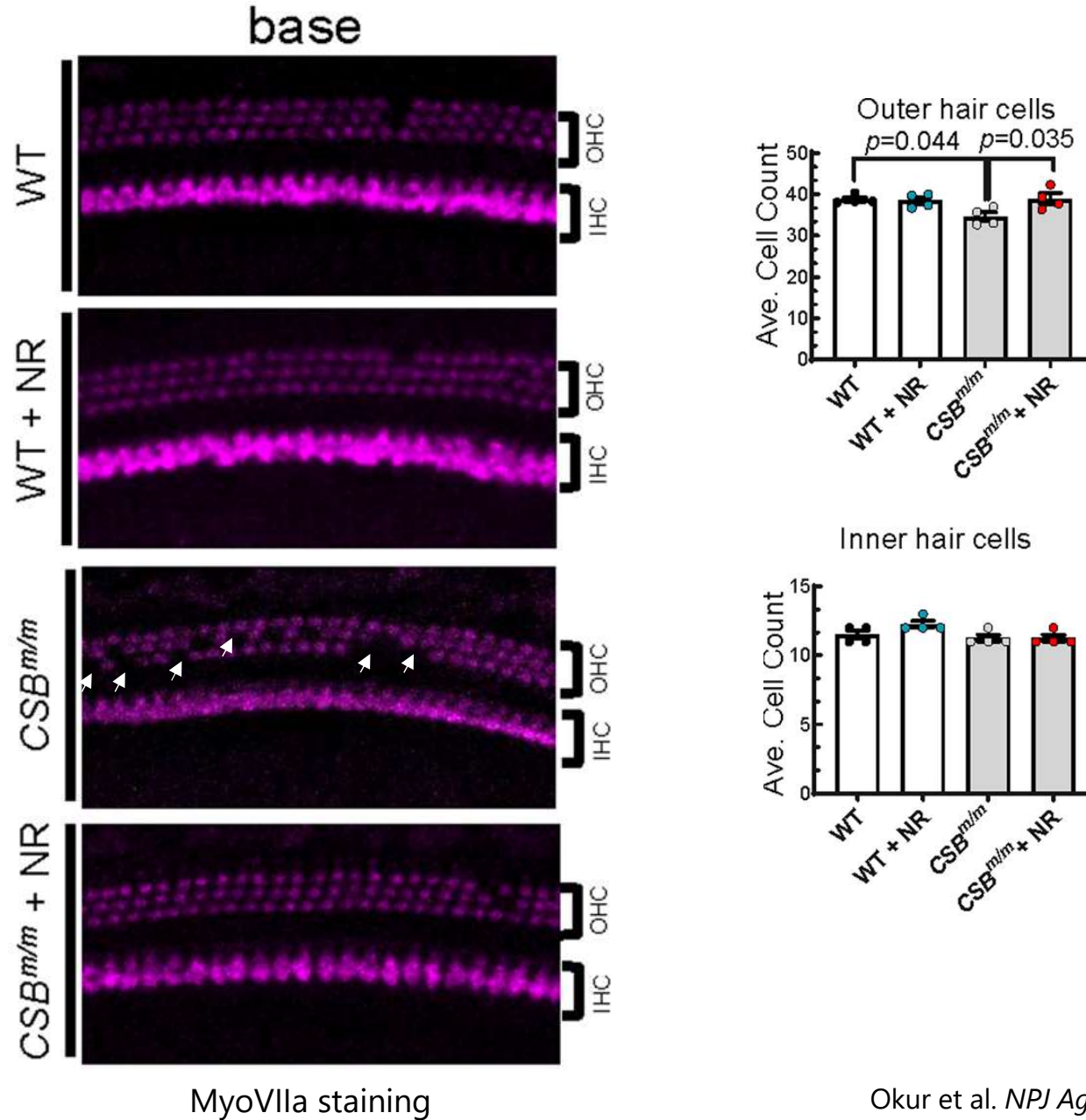
Hearing loss commonly arises from degenerative changes in the cochlea



1. Sensory cells
2. Spiral ganglion neurons
3. Synaptic Connectivity
4. Stria Vascularis

Image adapted from Jentsch *et al.* 2000 and Kujawa *et al.* 2009

NR treatment rescues other hair cells loss in CSB^{m/m} mice



Hearing loss commonly arises from degenerative changes in the cochlea

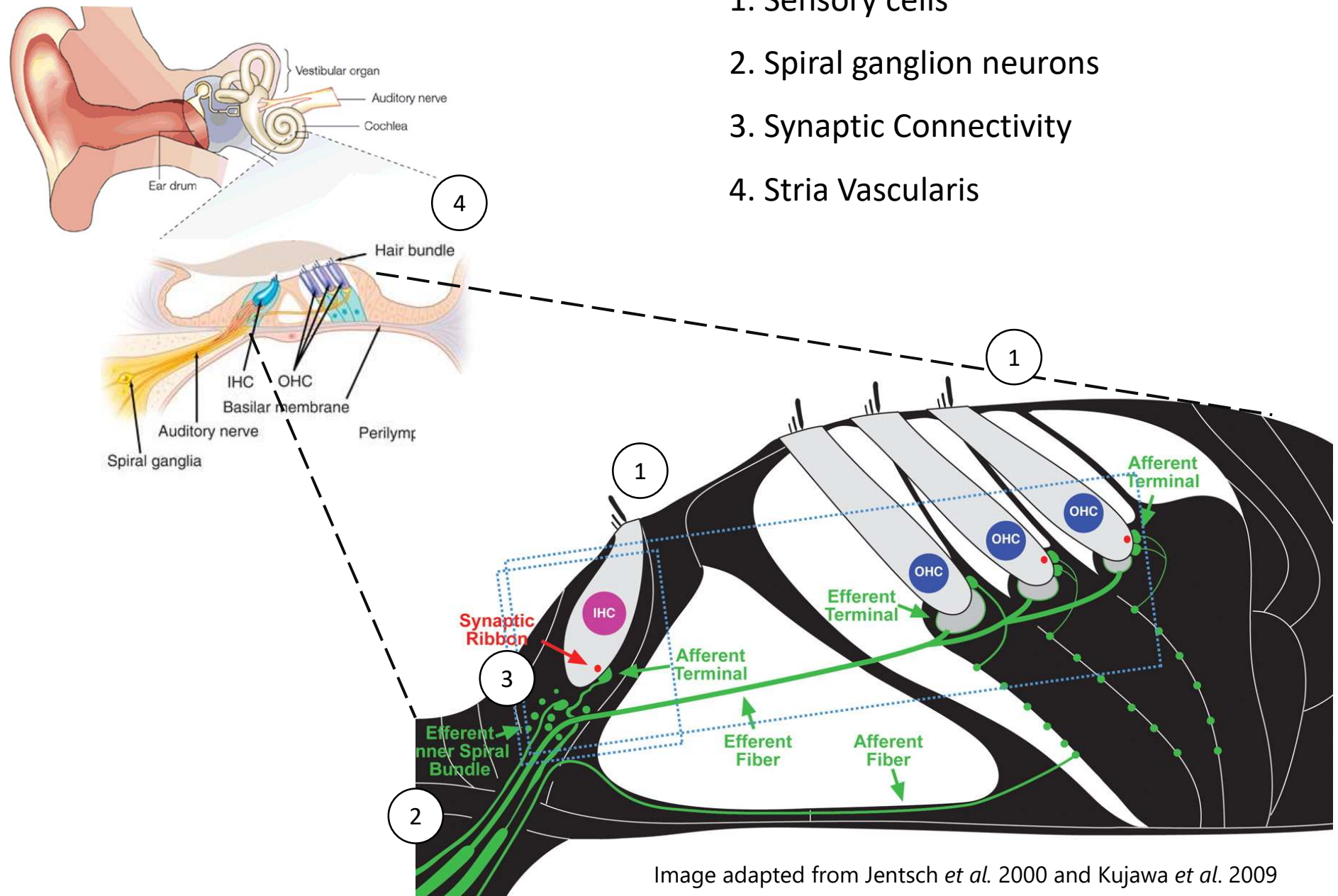
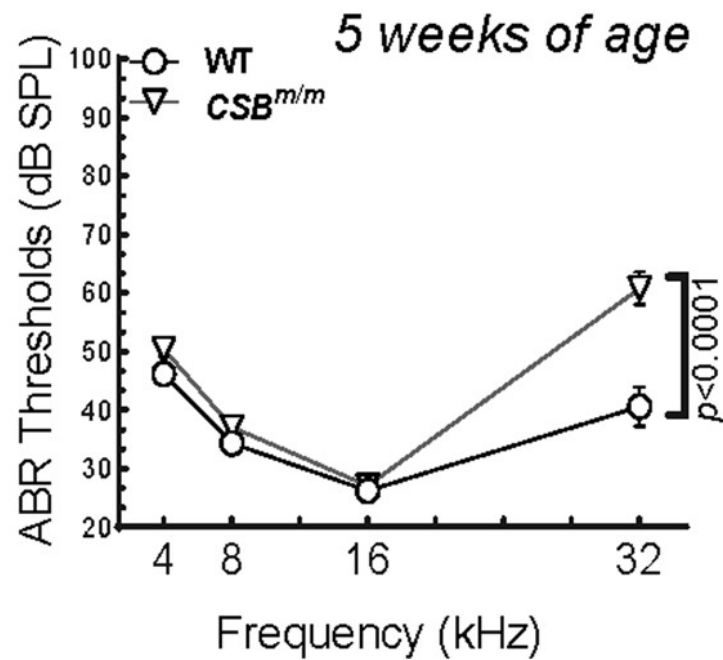
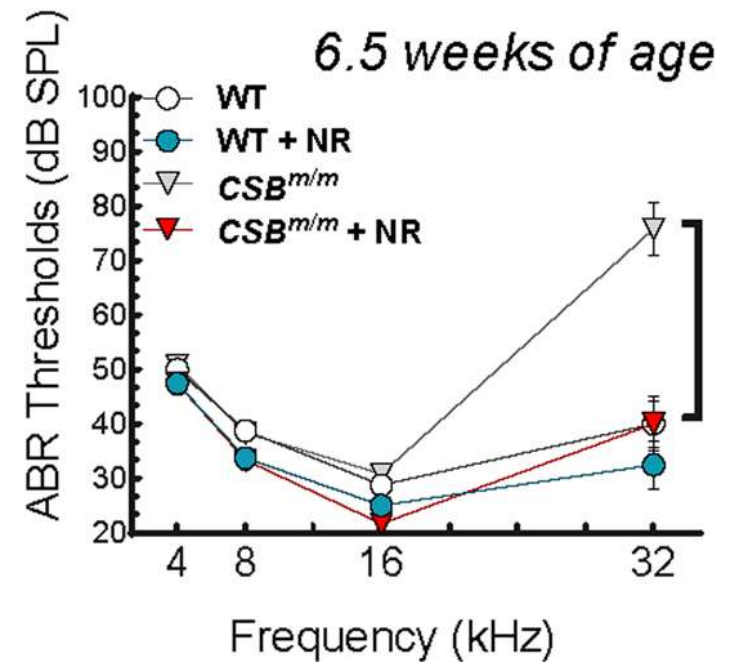


Image adapted from Jentsch *et al.* 2000 and Kujawa *et al.* 2009

NR treatment prevents the progression of hearing loss in $CSB^{m/m}$ mice



NR
Treatment
(10 days)



Hearing loss commonly arises from degenerative changes in the cochlea

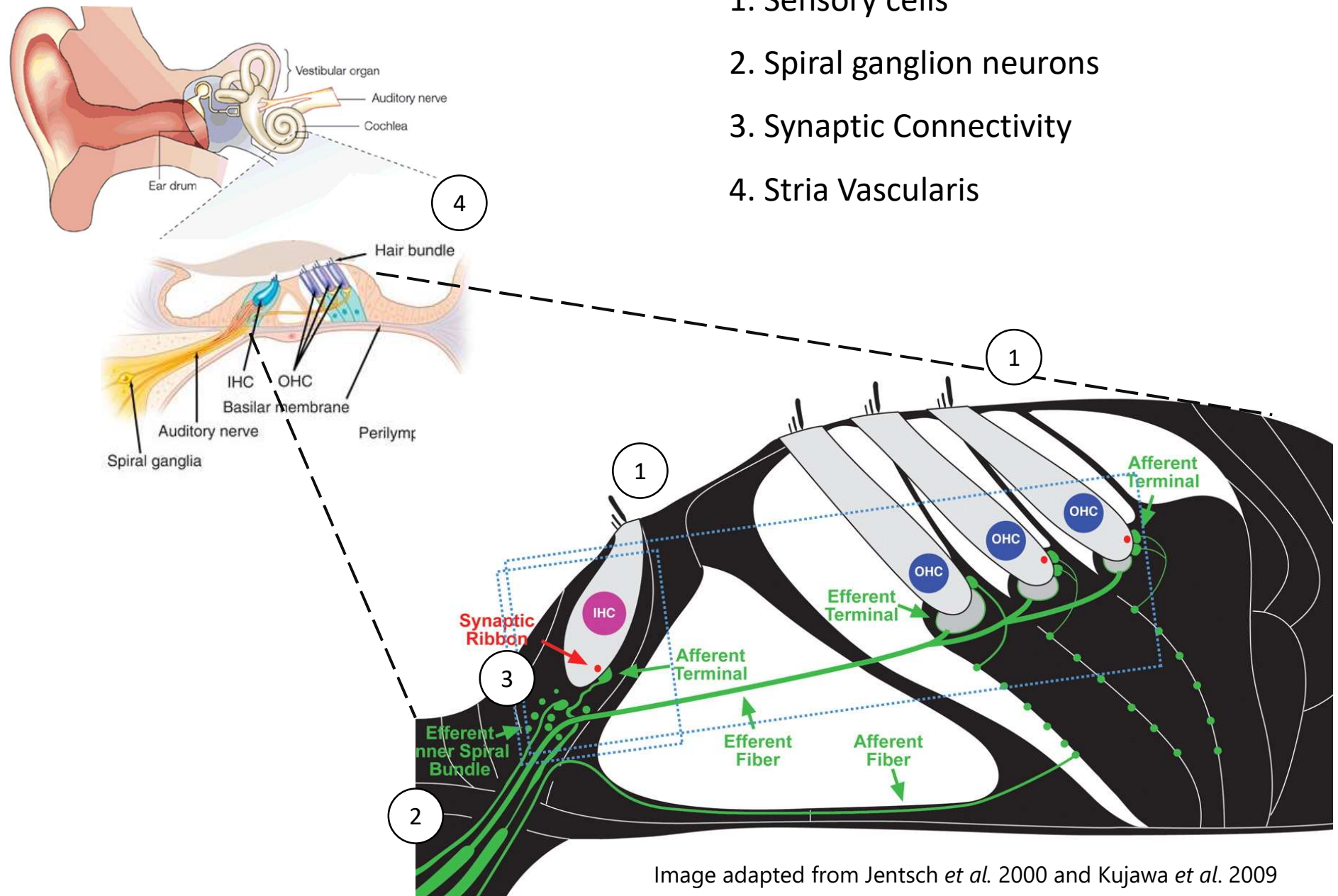
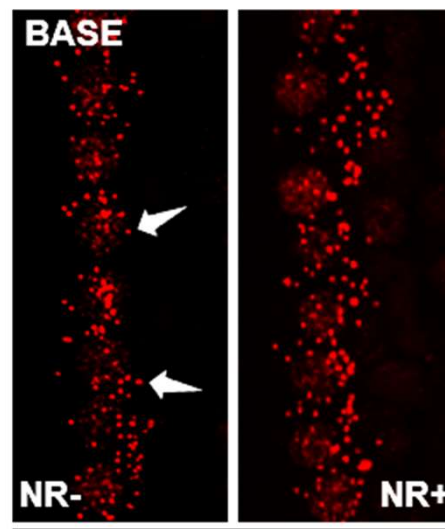
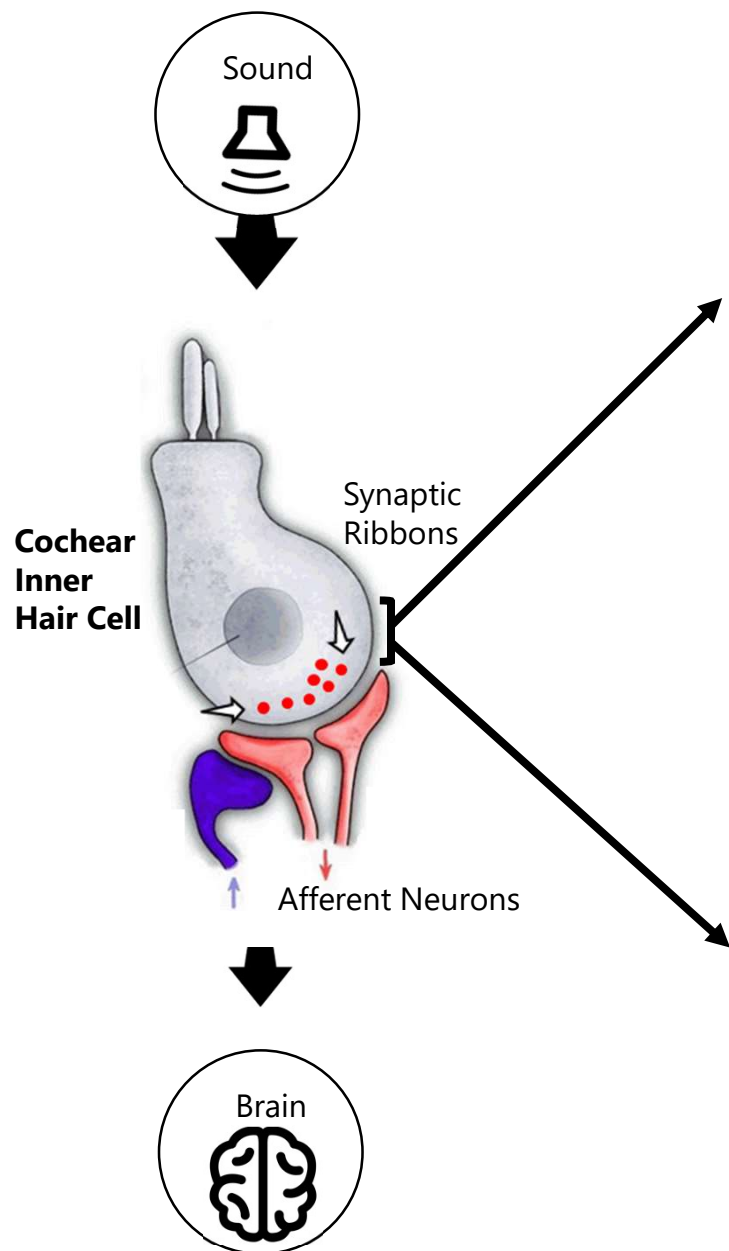
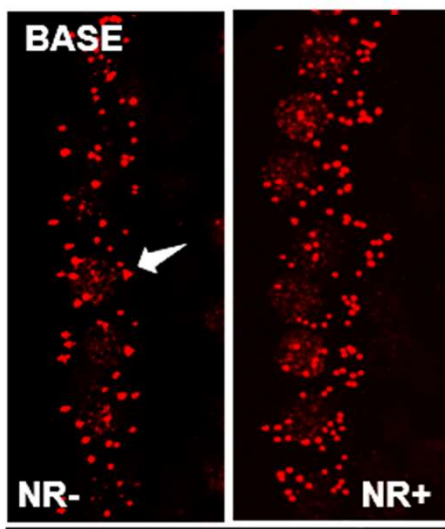


Image adapted from Jentsch *et al.* 2000 and Kujawa *et al.* 2009

NR enhances synaptic connectivity in the cochlea of $CSB^{m/m}$ mice

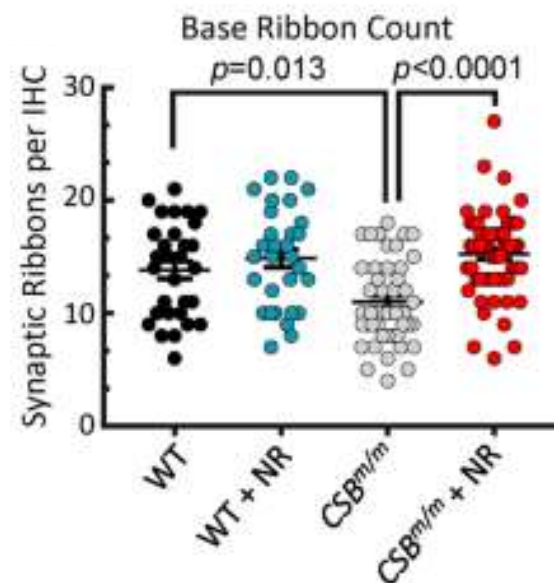


WT

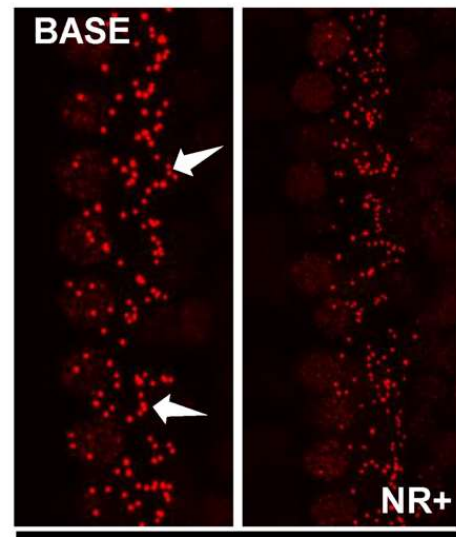
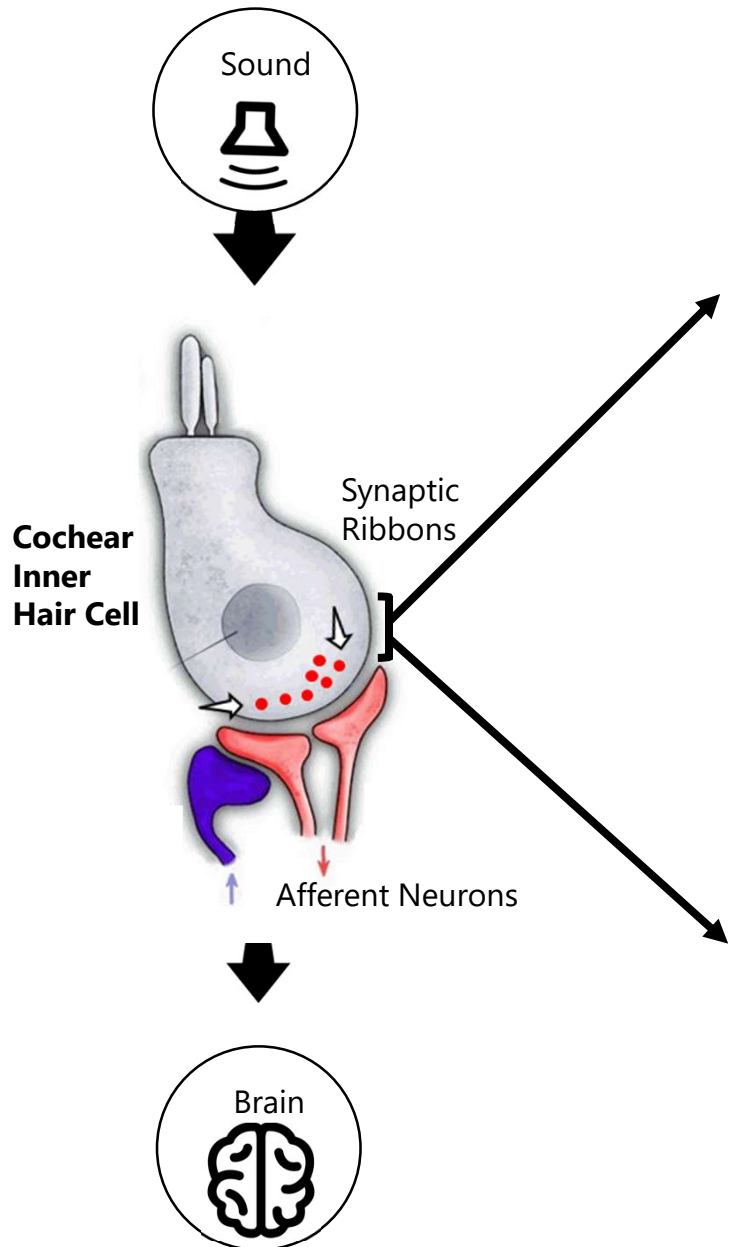


CSB

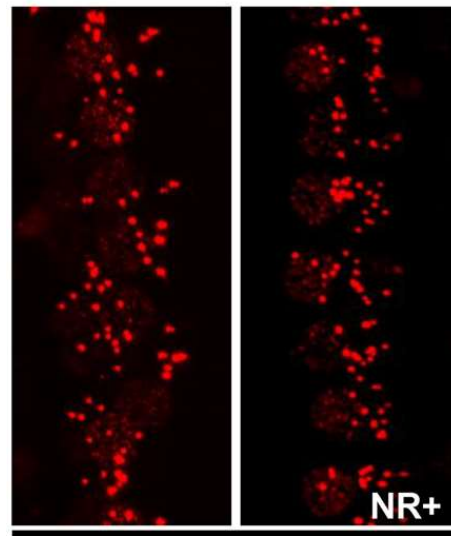
Anti-Ctbp2 : ribbon synapse marker



NR enhances synaptic connectivity in the cochlea of $CSA^{-/-}$ mice

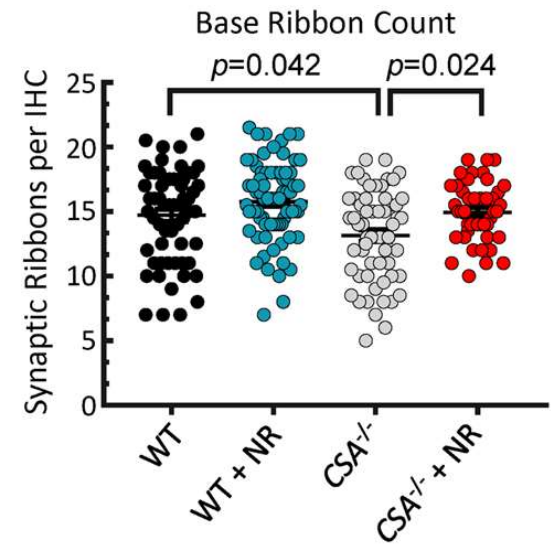


WT

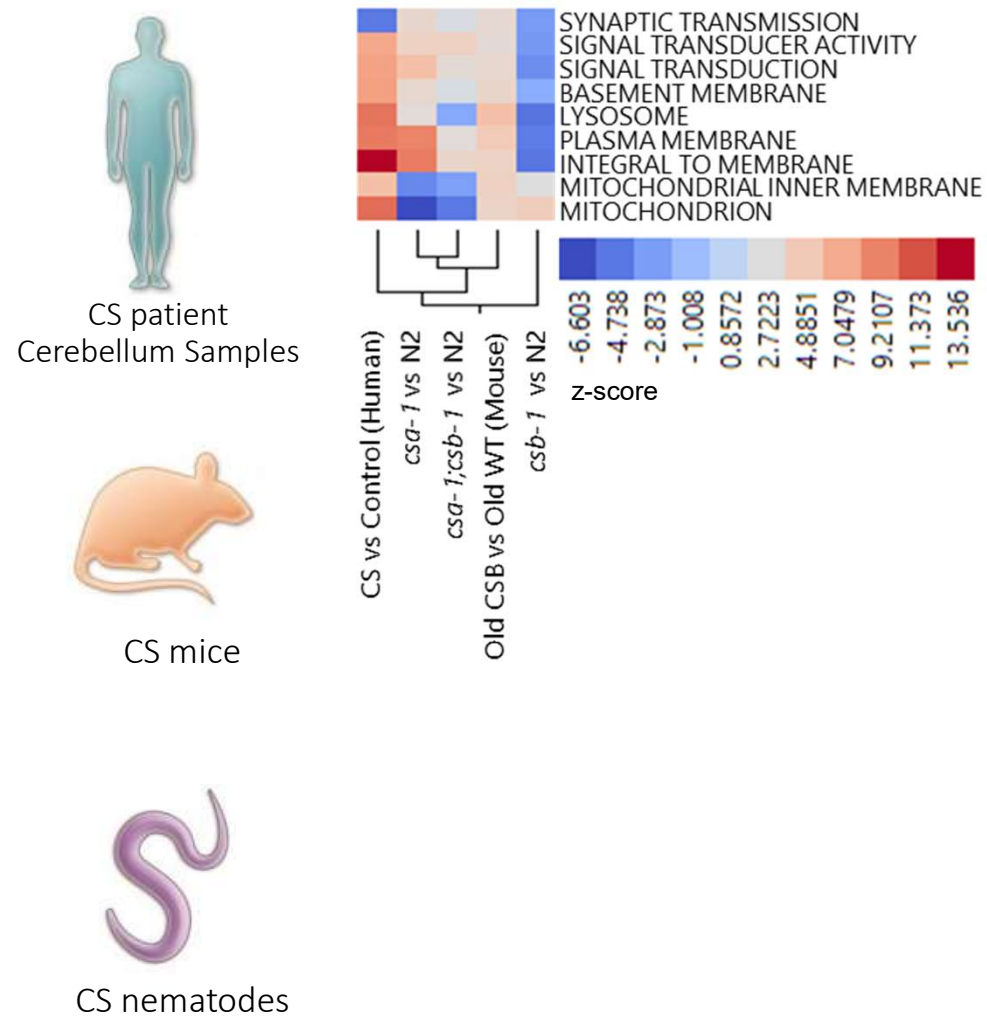


$CSA^{-/-}$

Anti-Ctbp2 : ribbon synapse marker



Synaptic Transmission were impacted in the models of CS and CS brain samples



Hearing loss commonly arises from degenerative changes in the cochlea

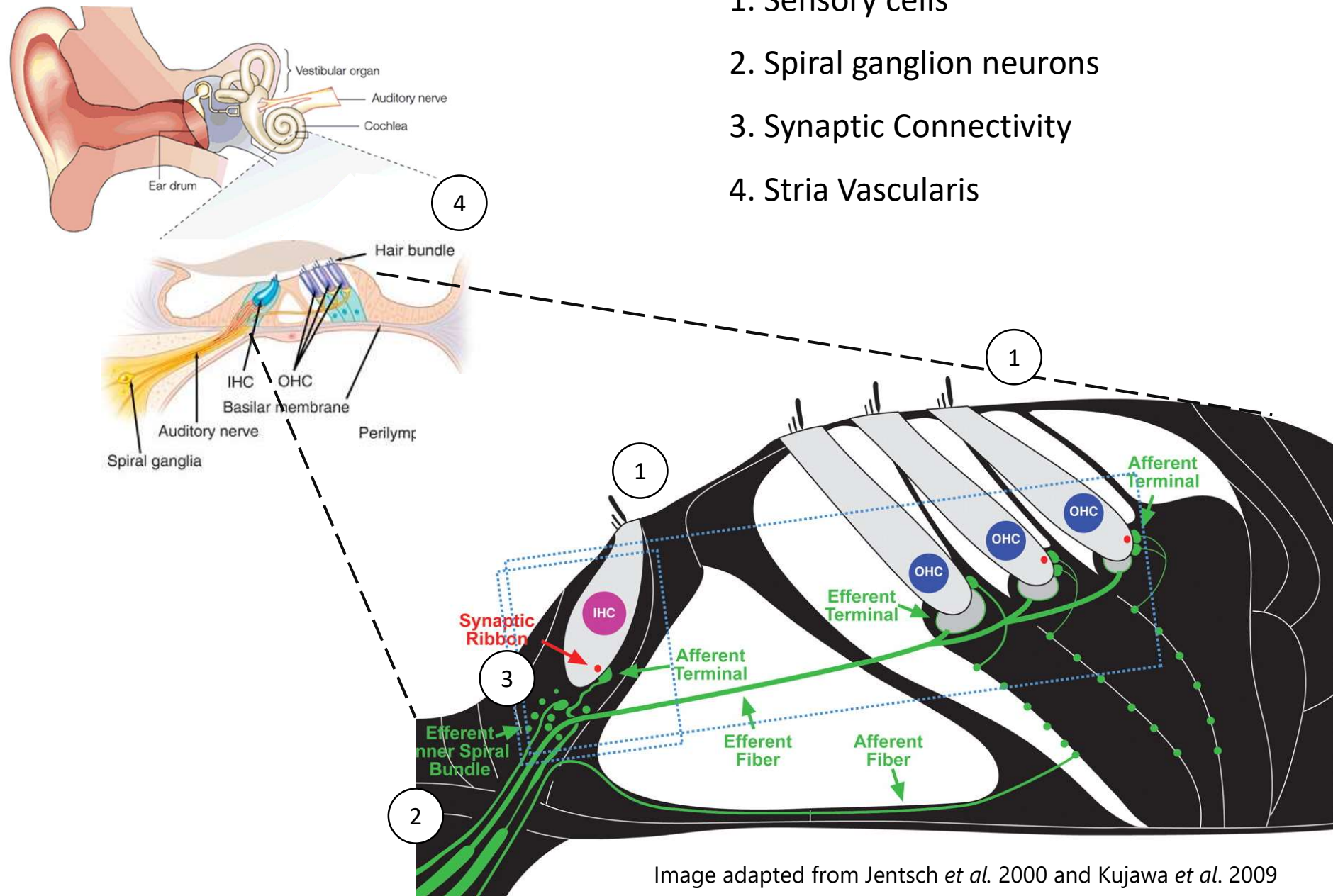
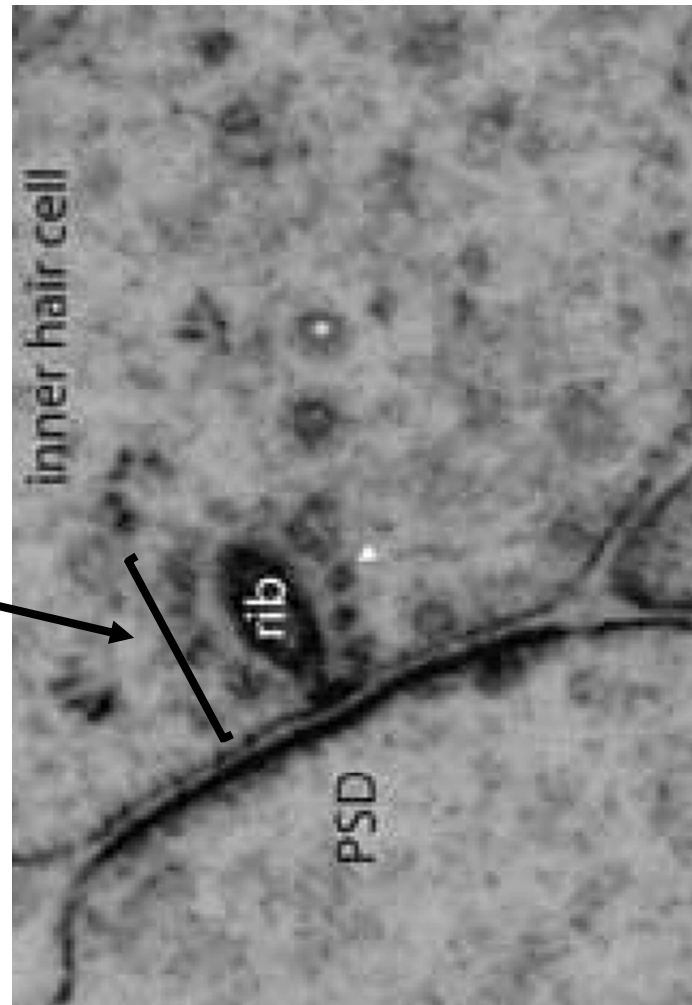
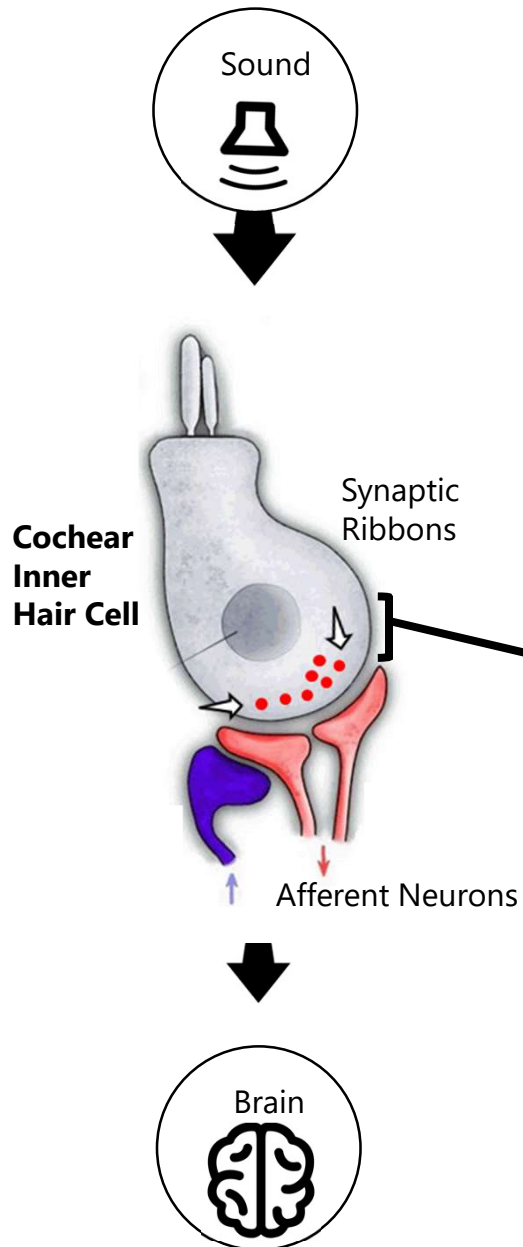


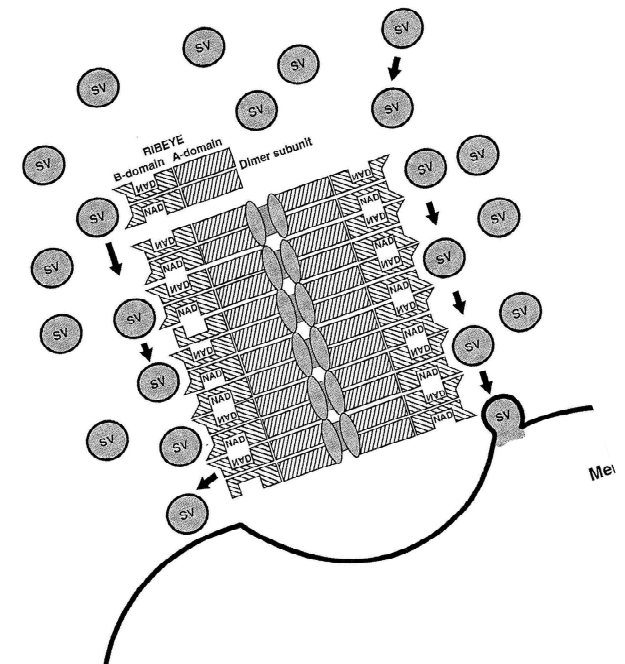
Image adapted from Jentsch *et al.* 2000 and Kujawa *et al.* 2009

Synaptic Ribbons

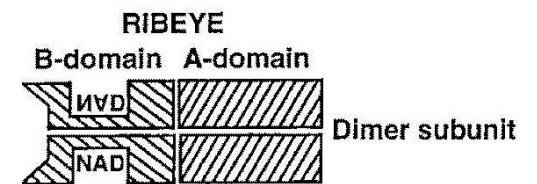


Hair cell ribbon synapse.
Nouvian et al. J. Membr Bio. 2006

Synaptic ribbon formation in inner hair cells



Ribeye Protein Structure



NAD(H) binding pocket

Current Research



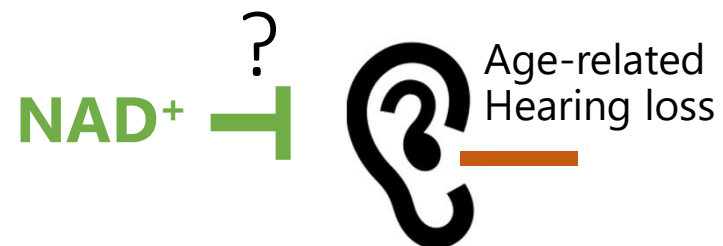
NAD⁺ supplementation prevents the progression of the hearing loss in CS

Okur et al, NPG Aging and Mechanisms of Disease, 2020

NAD⁺ declines with age in mice.

Schultz et al. Cell Metab, 2016

Zhang et al. Science, 2016



Conclusions

The gene classes of mitochondrial function and synaptic transmission are the common GO terms impacted in the models of CS

Mitochondrial terms in CS models are restored with NAD⁺ supplementation

NAD⁺ and NAD⁺/NADH levels were lower in CS cochlea

NAD⁺ supplementation via NR intervention prevents the progression of hearing loss in CS mice and restores outer hair cell loss in *CSB^{m/m}* mice.

NAD⁺ supplementation induces synaptic connectivity between sensory cells and afferent neurons in CS mice

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Thank you...