Hearing loss characterization in DBA/1JRj & DBA/2JRj mouse models

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Objectives

DBA/1JRj mice are widely used as a model of rheumatoid arthritis while **DBA/2JRj** mice are more studied in cardiovascular biology or neurobiology. However, due to their homozygous Cdh23^{ahl} mutation, a gene involved in mechano-transduction in the inner ear, **10 months old DBA/1JRj** mice **develop severe hearing loss** while **DBA/2JRj** exhibit **hearing defects starting at 2-3 months old**.

The objective of this study was to compare and better **characterize** hearing loss in early ages in these two **DBA mice strains**, from a **functional** and **histological** point of view. This study will help to assess the relevance of using DBA/1JRj mice as an *in vivo* model of rheumatoid arthritis-induced hearing loss.

Methods

Animals: 3 weeks old male DBA/1JRj, DBA/2JRj and CBA/JRj mice were used.

Body weight: Body weight was measured at Baseline (BL), $T_{+5WEEKS}$, $T_{+8WEEKS}$ and $T_{+10WEEKS}$

DPOAEs (Distortion Product Otoacoustic Emissions): DPOAEs were measured on both ears (one animal/one ear at a time) at $T_{+5WEEKS}$, $T_{+8WEEKS}$ and $T_{+10WEEKS}$ at 4 frequencies: 8, 16, 24 and 32 kHz at an intensity of 60 dB.

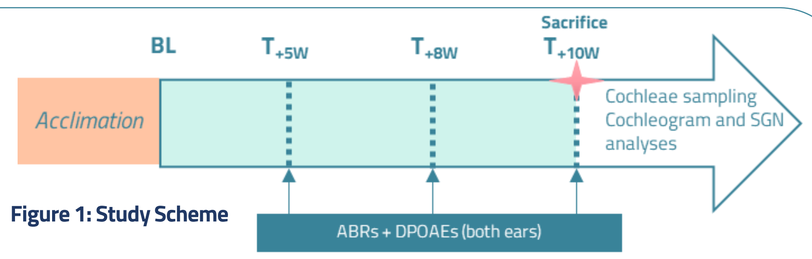


Figure 1: Study Scheme

ABRs (Auditory Brainstem Responses): ABRs were measured on both ears (six animals/one ear at a time) at $T_{+5WEEKS}$, $T_{+8WEEKS}$ and $T_{+10WEEKS}$ at 6 frequencies: 8, 16, 25, 32, 40 and 45 kHz.

Cytocochleograms: At $T_{+10\ WEEKS}$, cochleae (n=3-6/group) were sampled , fixed overnight in a 10% formalin solution, decalcified and then microdissected. Hair cells were immunolabeled with an anti-Myosin-VIIa antibody and images were acquired with a confocal microscope. Hair cell count was manually performed at 4, 8, 16, 25 and 32 kHz, using ImageJ software.

Spiral Ganglion Neurons (SGNs): At $T_{+10\ WEEKS}$, cochleae (n=3/group) were sampled, fixed overnight with a 10% formalin solution and decalcified. After paraffin embedding, cochleae were cross-sectioned (8 tissue sections spaced 20 µm apart), mounted on a microscope slide and stained with hematoxylin and eosin. Images were acquired with a NanoZoomer slide scanner. SGN count was performed at the apex, middle and base of each cochleae using the NanoZoomer software (NDP view).

Body weight results

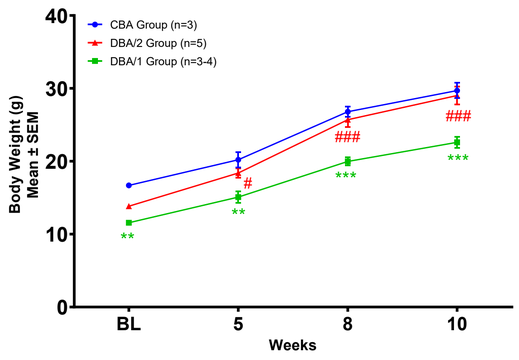


Figure 2: Mean Body Weight

Body weight increased from Baseline to $T_{+10WEEKS}$ for CBA, DBA2 and DBA1 mice. However, body weight was significantly lower in DBA1 mice, at all timepoints, compared to CBA mice and from $T_{+5WEEKS}$ to $T_{+10WEEKS}$, compared to DBA2 mice.

Two-way Anova followed by Tukey's post hoc test. *vs. CBA group, #vs. DBA/1 group; *p<0.05,** p<0.01, *** p<0.001

DPOAE results

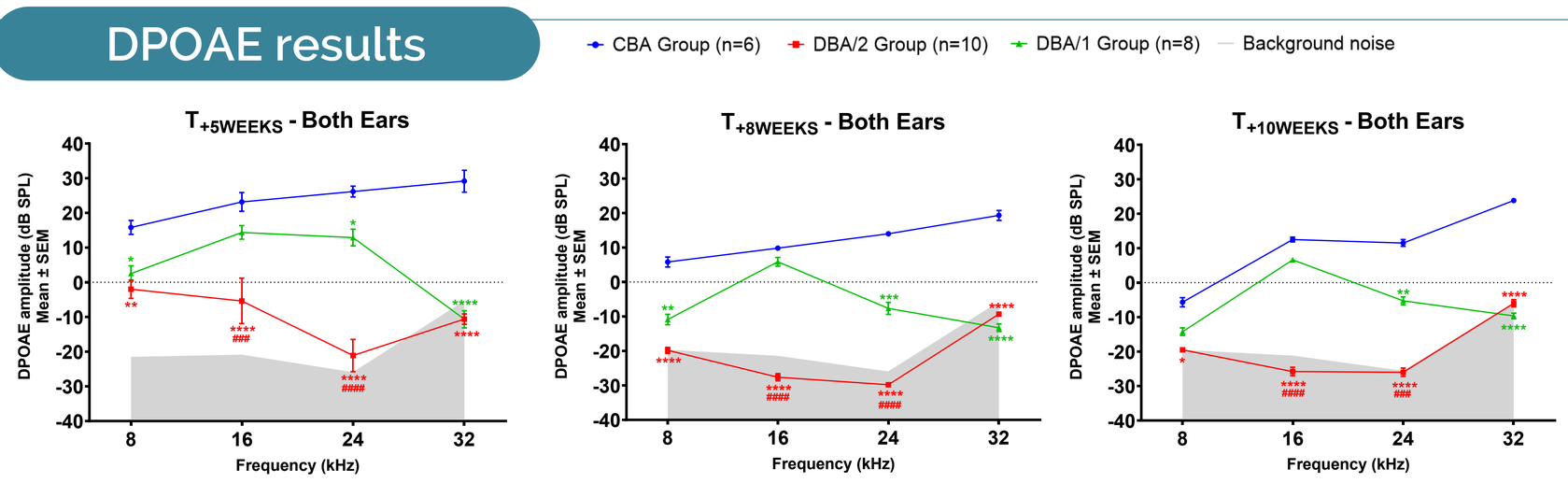


Figure 3: Mean DPOAE Amplitude at All Timepoints

All groups showed decreases in DPOAE amplitudes over time. In DBA1 mice, DPOAE were significantly decreased compared to CBA mice at 8, 24 and 32 kHz from $T_{+5WEEKS}$ to $T_{+8WEEKS}$ and at 24 and 32 kHz at $T_{+10WEEKS}$. In DBA2 mice, DPOAE amplitudes were further decreased at all timepoints, with significant reductions from 8 to 32 kHz, compared to CBA mice and at 16 and 24 kHz, compared to DBA1 mice, from $T_{+5WEEKS}$ to $T_{+10WEEKS}$.

Two-way Anova followed by Tukey's post hoc test. *vs. CBA group, #vs. DBA/1 group; *p<0.05,** p<0.01, *** p<0.001, **** p<0.0001

ABR results

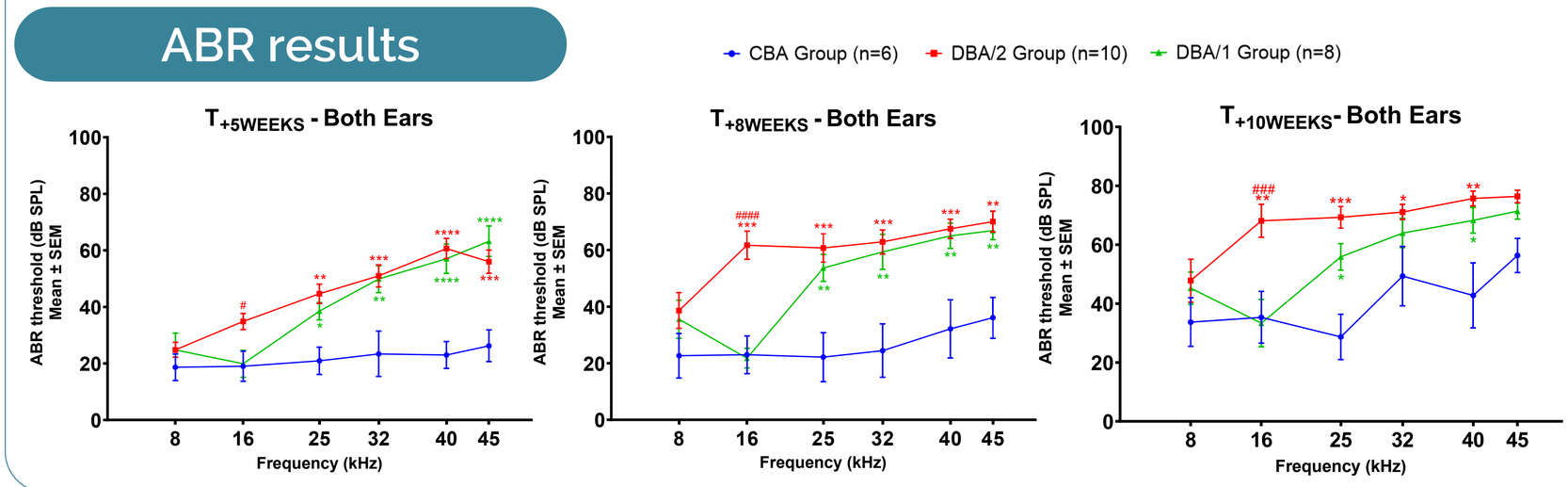


Figure 4: Mean ABR Thresholds at All Timepoints

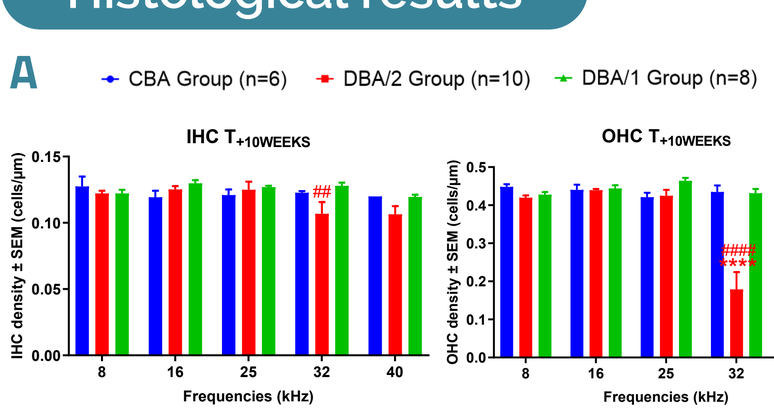
In CBA mice, ABR thresholds were consistent with normal hearing despite an increase at $T_{+10WEEKS}$.

In DBA1 mice, ABR thresholds were significantly increased from 25 to 45 kHz at $T_{+5WEEKS}$ and $T_{+8WEEKS}$ and at 25 and 40 kHz at $T_{+10WEEKS}$ compared to CBA mice.

In DBA2 mice, ABR thresholds were significantly increased at all timepoints compared to CBA mice: from 16 to 45 kHz, at $T_{+5WEEKS}$ and $T_{+8WEEKS}$ and from 16 to 40 kHz at $T_{+10WEEKS}$. Moreover, a significant increase was also observed at 16 kHz, compared to DBA1 mice, at all timepoints.

Two-way Anova followed by Tukey's post hoc test. *vs. CBA group, #vs. DBA/1 group; *p<0.05,** p<0.01, *** p<0.001, **** p<0.0001

Histological results



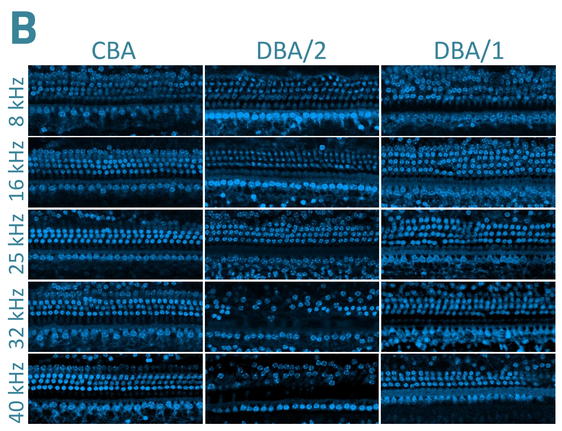
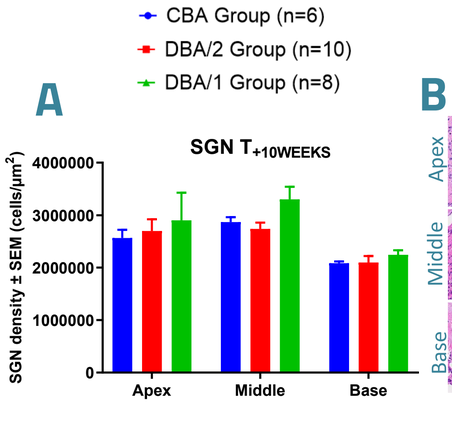


Figure 5: HC Density (A) and Corresponding Representative Images (B)

IHC density was similar at all frequencies for CBA, DBA2 and DBA1 mice, despite a slight decrease observed at 32 and 40 kHz compared to CBA and DBA1 mice, with statistical significance at 40 kHz compared to DBA1 mice. However, a significant decrease of OHC density was observed at 32 and 40 kHz in DBA2 mice compared to both CBA and DBA1 mice.

Two-way Anova followed by Tukey's post hoc test. *vs. CBA group, #vs. DBA/1 group; ** p<0.01, **** p<0.0001



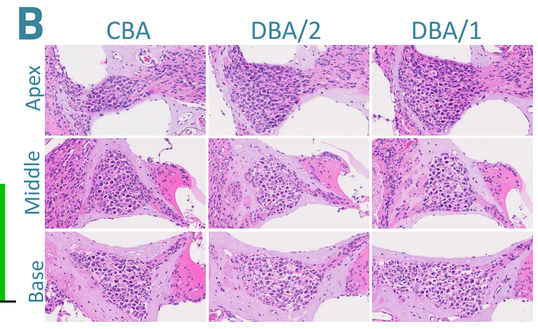


Figure 6: SGN Density (A) and Corresponding Representative Images (B)

SGN analysis indicated no significant difference between the different strains of mice, with a similar SGN density observed at the apex, middle and base of cochleae from CBA, DBA2 and DBA1 mice.

Two-way Anova followed by Tukey's post hoc test.

Conclusions

Both DBA/1JRj and DBA/2JRj mice exhibited **early-onset, progressive hearing loss** beginning as early as **5 weeks of age**. The hearing impairments were **more pronounced in DBA/2JRj mice**, as demonstrated by both functional and histological outcomes. Our results suggest that hearing loss observed in DBA/1JRj and DBA/2JRj mice occurred **earlier than previously reported in the literature** and should be carefully considered **for assessing hearing loss in rheumatoid arthritis models using DBA/1JRj mice.**