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Effect of changes in GABAergic inhibition on the development of tinnitus

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1 Introduction

Tinnitus is the perception of sound without an external source, often described as ringing, buzzing, or popping in the ears. It typically follows hearing loss caused by noise exposure, ototoxic drugs, infections, injuries, or genetic predisposition. Tinnitus can severely impact mental health and, in extreme cases, lead to depression or suicide. [1]
Neural correlates include increased spontaneous activity and synchrony of auditory neurons, likely due to reduced GABAergic inhibition. GABA-B receptors, composed of GB1/GB2 and KCTD auxiliary subunits, regulate neuronal excitability. KCTD proteins modulate receptor surface expression and kinetics. [2]

Aim: This study investigates how the absence of the KCTD16 protein affects GABA-B signaling, auditory function, and vulnerability to noise-induced hearing loss and tinnitus in mice.

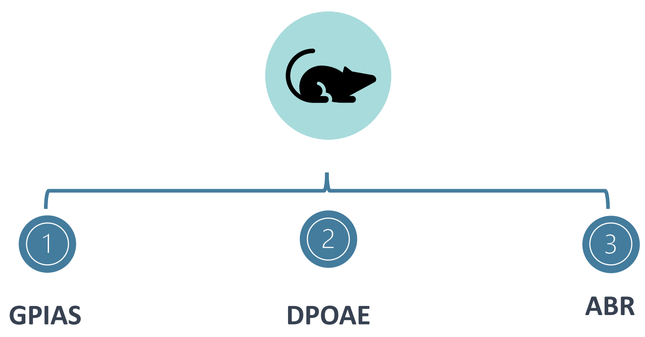
2 Methods

GPIAS (Gap Pre-pulse Inhibition of Acoustic Startle):
Tinnitus was assessed using the GPIAS method. Mice were placed in a soundproof chamber in wire cages on a pressure-sensitive platform. The startle response to acoustic stimuli was measured with and without a brief silent gap. GPIAS was calculated as: $GPIAS\ (\%) = (1 - [ASR\_gap\ /\ ASR\_no - gap]) \cdot 100.$

DPOAE (Distortion Product Otoacoustic Emissions):
Outer hair cell function was evaluated in anesthetized mice. DPOAEs were recorded via a microphone probe in the ear canal in response to two primary tones (*f2* = 4–40 kHz, *f2/f1* = 1.21, *L1/L2* = 70/65 dB SPL).

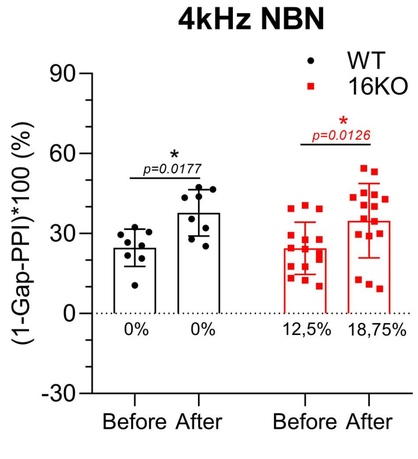
ABR (Auditory Brainstem Responses):
ABRs were recorded from anesthetized mice using subcutaneous electrodes. Sound stimuli (clicks and tones from 0–100 dB SPL) were presented in free-field conditions to determine hearing thresholds.

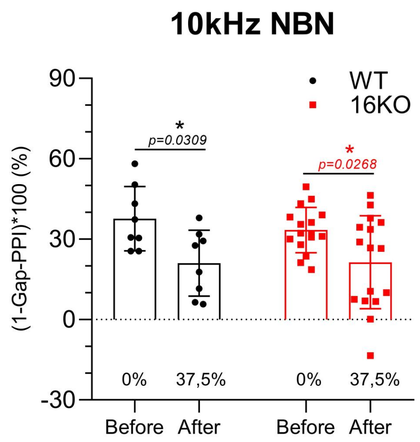
Noise Exposure:
Tinnitus was induced by exposing anesthetized mice to 10 kHz narrowband noise at 116 dB SPL for 1 hour.

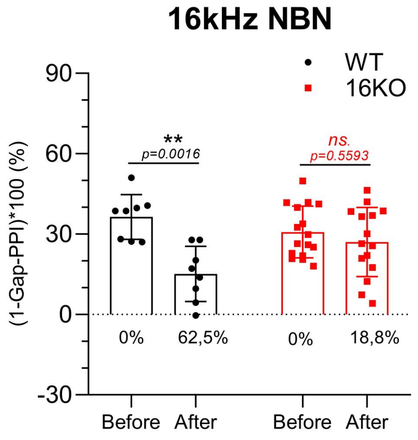


3 Results

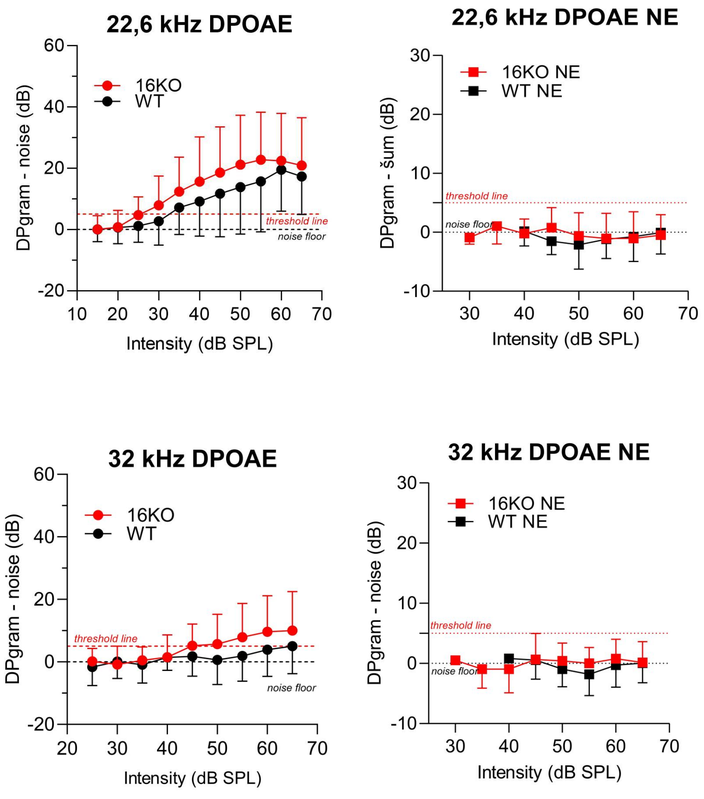
The following results show differences between the two groups of mice in their responses to acoustic stimuli. The first three graphs are a comparison of-GPIAS at 4, 10 and 16 kHz NBN before and after noise exposure.



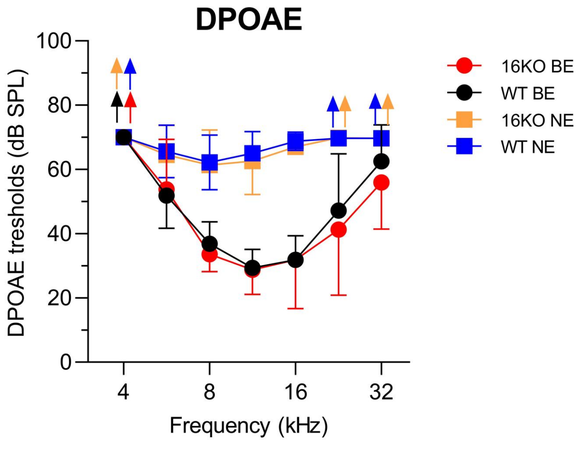




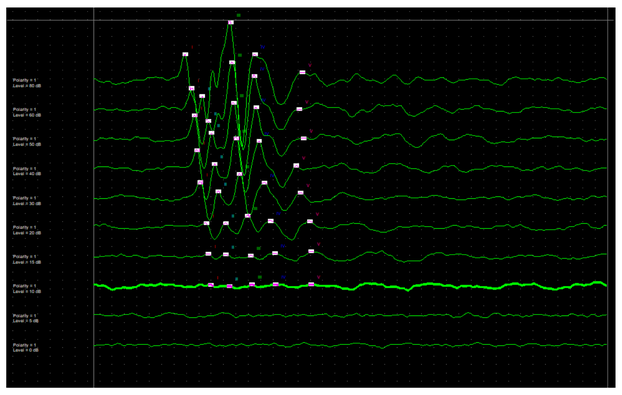
The second part of the results shows the difference between groups in DPOAE at higher frequencies. This difference was also confirmed for the DPOAE in response to the 8 kHz sound. *(NE = noise exposure)*

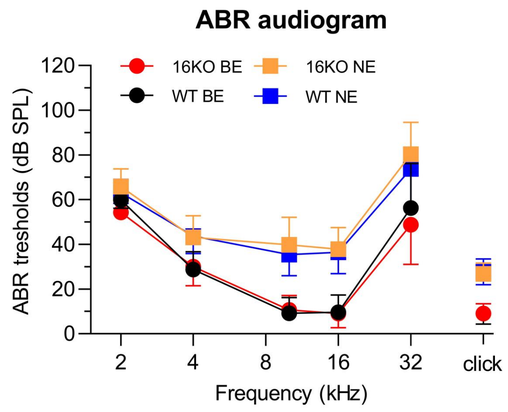


Elevation of hearing thresholds after noise exposure of animals was also observed using the DPOAE method. The arrows in the graph indicate that the response at a given frequency was not noticeable even at 70 dB. This intensity required to achieve a response is no longer increased due to the risk of further potential damage to the animal's hearing.



Further confirmation of mouse deafening is the shift in auditory thresholds shown by the ABR method. The figure below shows typical ABR waves from which the hearing threshold is subjectively determined.





4 Discussion

Tinnitus was assessed in two mouse groups using the GPIAS method. The 16KO group, lacking the KCTD16 protein, showed lower susceptibility to tinnitus after noise exposure compared to the WT group. ABR threshold shifts at low frequencies were also more pronounced in 16KO mice after deafening.
GPIAS was essential in detecting tinnitus both before and after acoustic trauma, revealing differences in inhibitory processing. **DPOAE** and **ABR** confirmed hearing loss, evident by reduced amplitudes and threshold elevations.

Tinnitus Findings:
Tinnitus was detected in 56% of 16KO mice and 75% of WT mice post-exposure. At 16 kHz, only 3 of 16KO mice developed tinnitus versus 5 of 8 WT mice. These results suggest a protective role of KCTD16 in processing high-frequency sounds.

Hearing Loss:
At 8 kHz, significant intra-group differences were observed after noise exposure. At higher frequencies (22.6–32 kHz), amplitude drops were evident across both groups, indicating hair cell dysfunction. ABR results showed threshold shifts at 4, 10, 16, and 32 kHz in both groups, with additional shifts at 2 kHz in 16KO mice, suggesting greater vulnerability to noise.

5 Conclusion

This study confirms that genetic differences between WT and 16KO mice influence their sensitivity to acoustic trauma, tinnitus development, and shifts in auditory thresholds. The GPIAS method was key in detecting tinnitus and identifying inhibitory differences between groups.
Supporting methods, DPOAE and ABR, confirmed hearing loss through reduced otoacoustic emissions and elevated thresholds. Notably, the 16KO group showed lower susceptibility to tinnitus at higher frequencies, suggesting that absence of the KCTD16 protein may enhance GABA-B receptor function.
These findings contribute to a better understanding of how genetic predispositions may shape vulnerability to tinnitus and auditory damage.

References

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