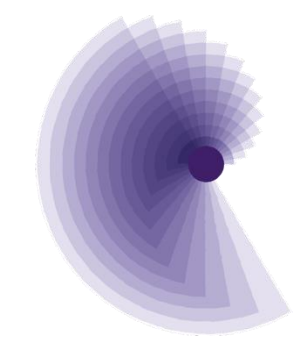




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Background

Clinical studies have identified a significant correlation between hearing loss and the progression of Alzheimer's disease (AD)^{1,2}. However, the causal relationship between the two remains poor understood. Amyloid-beta (A β), a hallmark of AD, may affect neurons responsible for hearing^{2,3}. Conversely, impaired cochlear function might also play a role in advancing AD⁴.

APP/PS1 mice are among the most frequently used transgenic mouse models in AD research. However, this model is on the C57BL/6 background, which suffers from early-onset hearing loss due to a mutation in *Cdh23* expressed in both the cochlea and brain⁵. To exclude the influence of the *Cdh23* mutation, we have "corrected" the *Cdh23* mutant. We aim to use our modified mouse model to investigate the relationship between hearing loss and memory impairment.

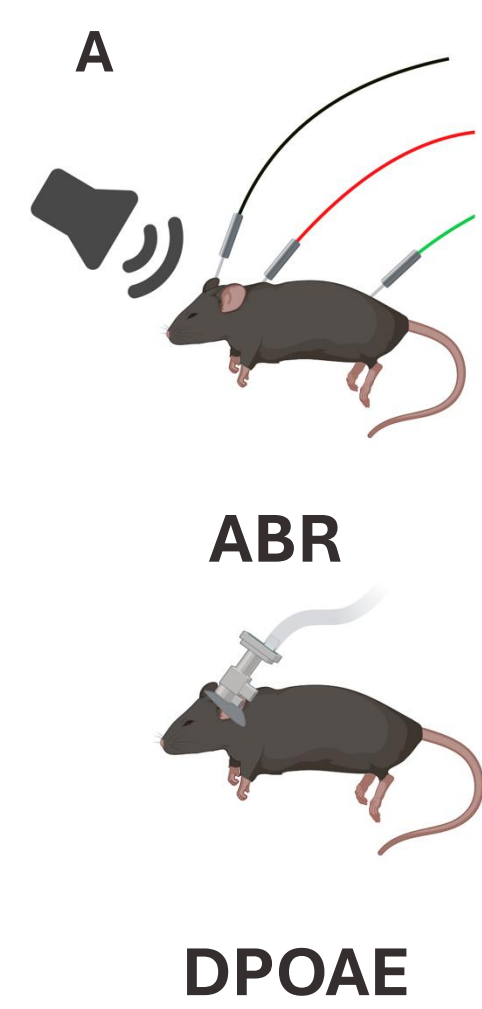
Methods

Animals To eliminate the influence of the mutant *Cdh23* (*Cdh23*^{753A}) allele, we crossed APP/PS1 of C57BL6/J background mice (AA-APP/PS1) with the B6.CAST-Cdh23Ahl+/Kjn strain (GG-WT), which carries the WT allele of *Cdh23*. We genotyped the offspring to confirm that the APP/PS1 and their WT littermate carry one copy of the WT *Cdh23* allele: GA-WT and GA-APP/PS1.

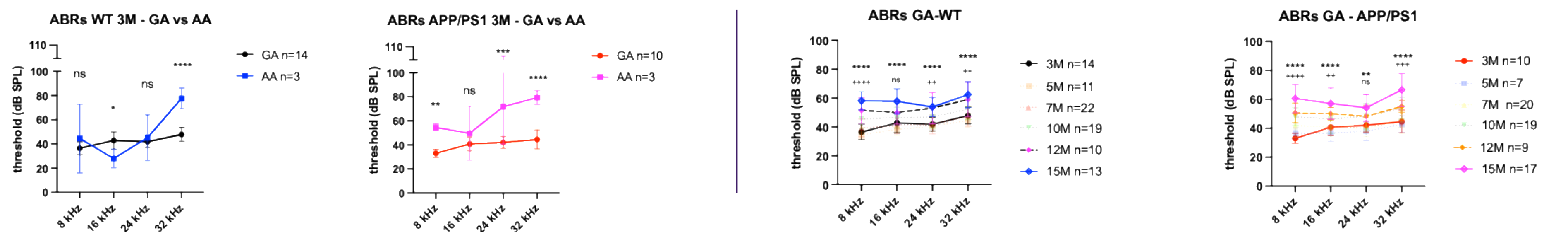
Hearing Mice had their hearing assessed with distortion product otoacoustic emission (DPOAE) to assess outer hair cell (OHC) and cochlear function and auditory brainstem response (ABR) to test neuron electrical signal transmission and function. Both tests are performed with the Tucker Davis Technologies system and analyzed in MATLAB. Testing began at 3 months of age and continued to be assessed at 5, 7, 10, 12, and 15 months of age.

Behavior Behavior was assed with the use of the open field test (OFT) to measure general locomotion and acclimation to the testing environment. Novel object recognition (NOR) was used to test working memory ability. Y-maze testing was used to assess spatial memory ability. OFT and NOR testing began at 7 months of age and continued to 10, 12, and 15 months of age. Y-maze testing began at 15 months of age. The use of ANYmaze software was used to test and analyze results of OFT, Y-maze, and NOR. NOR was additionally manually scored via use of a stopwatch timer to determine time spent investigating objects to prevent false positive results. Investigation criteria was defined as touching, pressing against, smelling, and looking at the object.

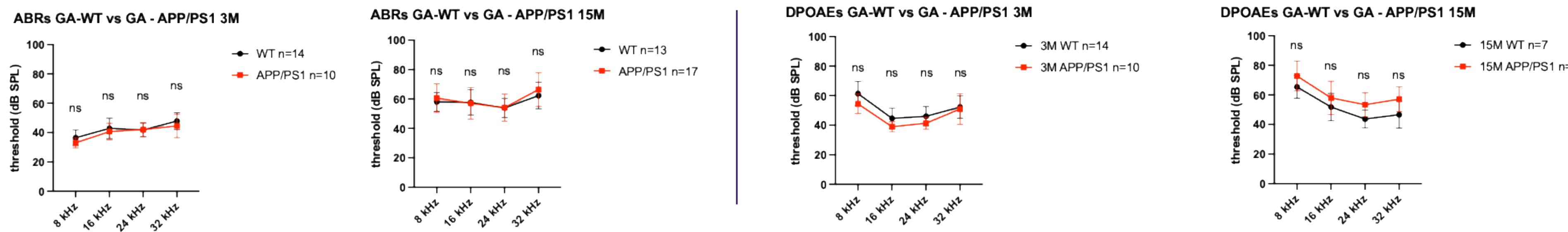
Onset of age-related hearing loss is delayed in both APP/PS1 and WT mice after CDH23 is corrected



B. AA mice exhibit high-frequency hearing loss by 3 months, while both GA-WT and GA-APP/PS1 mice experience age-related hearing loss beginning at 12 months.



C. There is no difference in hearing between GA-WT mice and GA-APP/PS1 mice measured by both ABR and DPOAE ranging from 3- to 15-months.



D. GA-APP/PS1 mice show increased thresholds at 15 months, while GA-WT mice have no differences between 3 and 15 months, as measured by DPOAE.

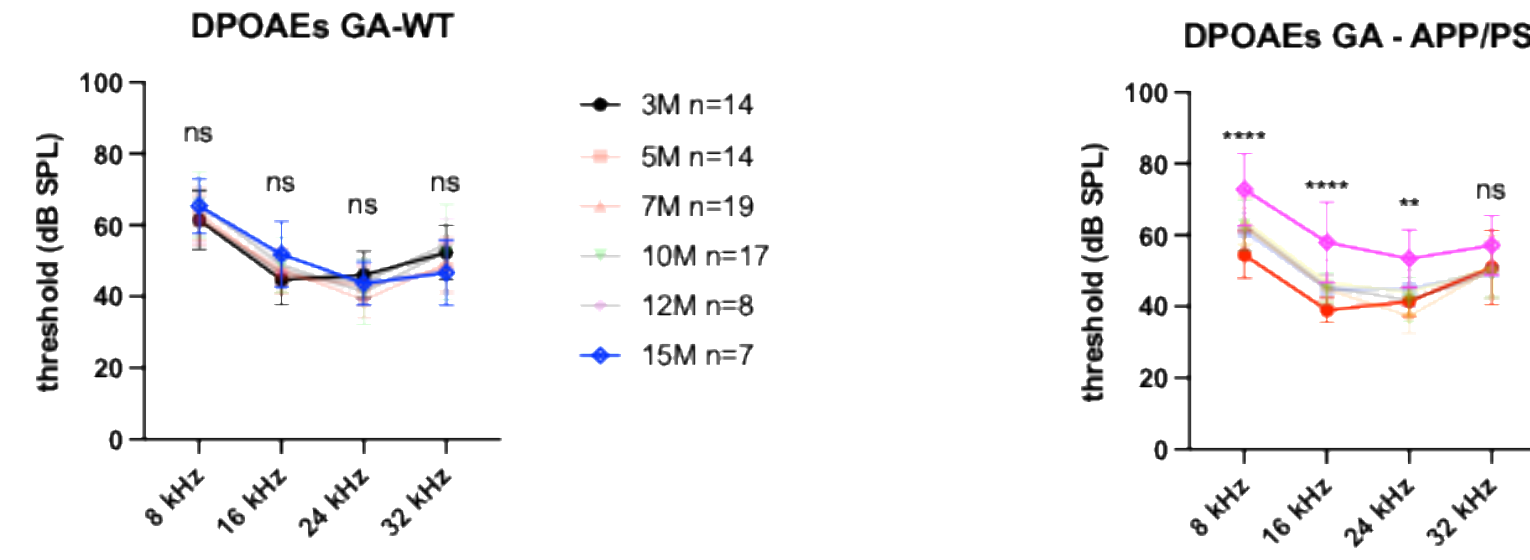


Fig 1. A. A sketch of ABR and DPOAE setup. **B.** Both AA-WT and AA-APP/PS1 start experiencing ABR threshold shifts at 32 kHz at 3 months of age compared to GA-mice, which only begin to have ABR threshold shifts at 12 months of age indicated by +. At 15-months, both GA-WT and GA-APP/PS1 mice show increased ABR thresholds at all frequencies indicated by *. **C.** From 3- to 15 months, GA-WT and GA-APP/PS1 show no difference in hearing in both ABR and DPOAE testing. **D.** GA-WT mice have no change in DPOAEs as they age while GA-APP/PS1 mice do exhibit threshold shifts at 15 months of age. The number of samples and means $\pm$ SD are shown. Analysis was performed with two-way ANOVA. + for 3M vs 12M and * for 3M vs 15M. ns = not significant, +, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$. M: month.

APP/PS1 mice in which CDH23 is corrected do not exhibit significant behavioral changes until 15 months of age

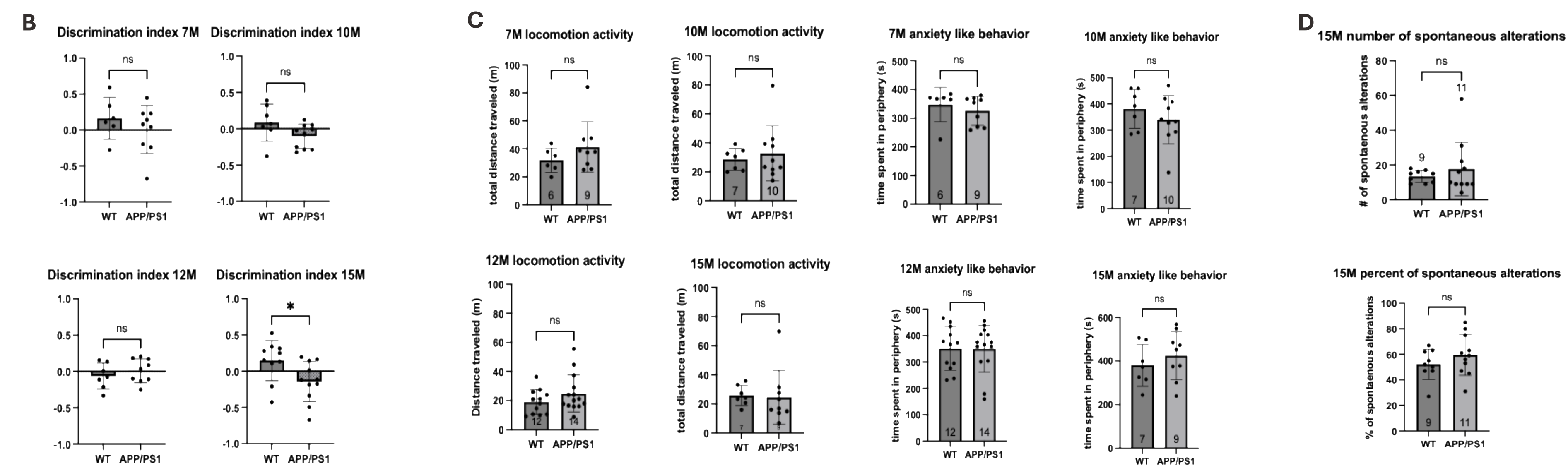
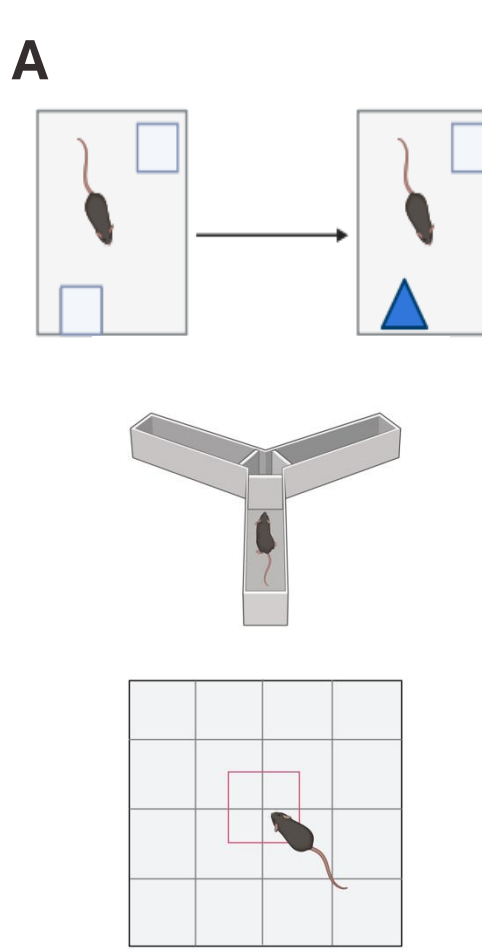


Fig 2. A. A graphic sketch of NOR, Y-maze, and OFT setup. **B.** NOR data. GA-APP/PS1 mice did not show an impaired performance in NOR until 15 months. **C.** OFT data: There is no difference between GA-WT and GA-APP/PS1 mice in both locomotion activity and anxiety like behavior from 7 to 15 months. **D.** Y-maze Data. There is no difference between GA-WT and GA-APP/PS1 mice in spatial working memory at 15 months. The number of samples and means $\pm$ SD are shown. Analysis was performed with Student's *t*-test. ns = not significant, *, $p < 0.05$.

Discussion and Conclusion

Both AA-WT and AA-APP/PS1 mice exhibit high-frequency hearing loss associated with the mutant *Cdh23* allele by 3 months of age. In contrast, GA mice do not exhibit age-related hearing loss until 12 months of age, suggesting that correction of the *Cdh23* mutation delays the onset of hearing loss.

Although there is no significant difference in hearing between GA-WT and GA-APP/PS1 mice from 3 to 15 months, older GA-APP/PS1 mice do show a significantly increased threshold compared to younger GA-APP/PS1 mice, as measured by DPOAE. In contrast, GA-WT littermates do not experience threshold changes in DPOAE with age. The mechanism behind the decreased OHC function remains unclear. One possibility is that A β may enter the cochlea from the brain and impair OHC function.

AA-APP/PS1 mice typically begin to show memory decline around 7 months⁶, while GA-APP/PS1 mice show delayed onset of memory decline, suggesting that hearing loss may have a more significant impact on AD progression than previously thought. Since CDH23 expression is not limited to the cochlea, further research is necessary to understand its interactions with hearing loss and Alzheimer's disease.

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Acknowledgements

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