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Temporal progression of auditory brainstem dysfunction and behavioral phenotypes in mouse models of tauopathy

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Abstract

Cognitive decline in Alzheimer's disease and related dementias (AD/ADRD) frequently co-occurs with motor and sensory processing impairments. While auditory abnormalities were reported in beta-amyloid-based models before cognitive decline, the effects of tau pathology on auditory circuit function remain poorly understood. The PS19 mouse model expressing the P301S tau mutation exhibits tau pathology associated with frontotemporal dementia. When combined with the humanized ApoE ε4 allele, these mice show accelerated tau accumulation and neurodegeneration. We investigated the temporal progression of auditory and behavioral deficits in PS19 and ApoE4/PS19 mice of either sex at 3 and 7 months. Auditory processing was assessed using auditory brainstem response (ABR) recordings, while anxiety-like and motor behaviors were evaluated using the elevated plus maze (EPM) and rotarod assays. PS19 mice exhibited auditory pathway dysfunction at 3 months, characterized by reduced and delayed neural responses to acoustic stimuli. These auditory deficits occurred without detectable behavioral changes in anxiety- or motor-related measures. By 7 months, cochlear-nucleus-derived ABR activity shifted from an initial reduction to elevation relative to controls, suggesting compensatory hyperexcitability. In contrast, ApoE4/PS19 mice showed reduced ABR amplitudes and shortened response latencies that persisted with age, accompanied by altered anxiety-like behavior and motor performance. Together, these results demonstrate that auditory pathway dysfunction may represent an early marker of tau pathology preceding behavioral impairment. The divergent temporal patterns of ABR alterations across genotypes suggest that genetic context, particularly ApoE4, modulates the progression of tau-driven circuit dysfunction, highlighting ABR as a potential noninvasive biomarker for early tau-related neurodegenerative disease.

Significance Statement

Early detection of Alzheimer's disease and related dementias remains a major clinical challenge. This study identifies auditory pathway dysfunction as an early, measurable marker of tau pathology, preceding detectable behavioral impairments in mouse models with abnormal auditory brainstem responses (ABRs) appeared months before anxiety or motor behavioral changes. Genetic background also significantly influenced disease progression, with the ApoE4 allele altering both the timing and nature of auditory and behavioral phenotypes. Together, these findings demonstrate that tau pathology disrupts auditory processing at early disease stages and suggest that ABR-based measures as sensitive, non-invasive approach for early detection and monitoring of tau-related neurodegeneration.

Introduction

Alzheimer's disease and related dementias (AD/ADRD) represent a complex group of neurodegenerative disorders characterized by progressive cognitive decline, affecting millions of individuals worldwide. While memory impairment remains the most recognized symptom, growing evidence suggests that sensory processing deficits, particularly in auditory function, may serve as early indicators of disease progression (Zhang et al., 2023).

The pathological hallmark of AD/ADRD is characterized by the accumulation of misfolded proteins, notably tau neurofibrillary tangles and beta-amyloid plaques, which disrupt normal neuronal function and drive neurodegeneration. Tau pathology is strongly associated with frontotemporal dementia and correlates closely with the severity of cognitive decline (Gulisano et al., 2018). The apolipoprotein E (ApoE) ε4 allele is the most significant genetic risk factor for late-onset Alzheimer’s disease, linked to earlier onset and more rapid disease progression (Jun et al., 2011).

Recent studies have highlighted a relationship between sensory deficits and cognitive dysfunction, raising interest in sensory markers as potential early indicators of neurodegeneration. In AD mouse models, auditory processing abnormalities have been shown to precede learning and memory impairments, suggesting their potential utility as early biomarkers for disease progression (Liu et al., 2020; Weible et al., 2020; Zhang et al., 2023).

The PS19 transgenic mouse, which expresses the P301S humanized tau mutation, is a well-established model of tauopathy, displaying features of frontotemporal dementia, including neurofibrillary tangles and progressive neurodegeneration (Patel et al., 2022). When combined with the human ApoE ε4 knock-in allele, this model exhibits accelerated tau deposition and pronounced atrophy, with pathology detectable as early as 3 months of age (Williams et al., 2022; Seo et al., 2023).

Although beta-amyloid-based models such as APP/PS1 and 5xFAD mice have been extensively studied for auditory abnormalities, the effects of tau pathology on auditory processing remain understudied (Weible et al., 2020; Weible and Wehr, 2022). Understanding how tau-mediated neurodegeneration disrupts auditory pathways could reveal new mechanistic insights and inform early diagnostic tools. In addition to sensory deficits, changes to anxiety-related behaviors are prevalent in AD/ADRD and may exacerbate the effects of sensory dysfunction, further impairing cognitive and functional outcomes (Samaey et al., 2019).

This study addresses these gaps by investigating age-related changes in auditory processing and behaviors in PS19 and ApoE4/PS19 mice relative to their controls. Using auditory brainstem response (ABR) testing, elevated plus maze (EPM), and rotarod assessments, we evaluated mouse models at 3-4, 6-7, and 9-10 months to characterize the progression of auditory deficits and assess how the interaction between tauopathy and ApoE ε4 modulates disease trajectory (Figure 1). ABR testing provides an objective assessment of auditory pathway integrity independent of behavioral confounds, enabling detection of neural

dysfunction that may precede observable behavioral deficits (Riley et al., 2021; Tanaka et al., 2023).

Behavioral characterization focused on anxiety-like behavior and motor function rather than hippocampus-dependent memory tasks reflects the neuropathological staging of the PS19 model: tauopathy initiates in brainstem and subcortical structures, including circuits mediating motor output and limbic function, well before substantial hippocampal involvement, and reliable cognitive deficits do not emerge until 8-9 months (Scattoni et al., 2010; Ahmad et al., 2021).

Our results show that PS19 mice exhibit early-onset auditory abnormalities by 3 months of age, characterized by reduced peak amplitudes across the subcortical auditory pathway and prolonged peak and interpeak latencies, indicating impaired neural transmission. These deficits persisted at 7 months, with continued latency delays despite partial amplitude normalizations. In contrast, ApoE4/PS19 mice show a distinct pattern, with significant amplitude reduction and latency shortening at 3 months, persisting to 7 months. Behaviorally, PS19 mice maintained normal anxiety-like behavior and motor function at both ages, whereas ApoE4/PS19 mice displayed changes in anxiety-like behavior as early as 3 months and motor performance at 7 months compared to controls. These findings reveal distinct trajectories of auditory dysfunction across genotypes, highlighting auditory processing measures as potential indicators of disease progression in tauopathy models.

Methods

Animals

All experiments were conducted according to the National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee

(IACUC, protocol # AUP-22-163) and the Institutional Biosafety Committee (IBC, protocol # BUA-R100) of the University of California, Irvine.

In this study, approximately 80 mice were used across three age groups. PS19 mice (JAX #008169; B6C3F1/J hybrid background) and ApoE4 knock-in mice (provided by the La Spada Laboratory, UC Irvine) were maintained as separate colonies on a hybrid B6C3F1/J background, with all mice heterozygous at the Cdh23 locus. ApoE4/PS19 mice were generated within their own colony and were not intercrossed with the PS19-only line. WT mice are P301S+ littermates from the respective colonies.

The younger cohort (3-4 months) consisted of 8 wild-type (WT) mice, 9 PS19 mice, 11 ApoE4 knock-in (ε4 allele) mice, and 9 ApoE4/PS19 double-transgenic mice. The older cohort included 16 to 17 mice per genotype group: WT, PS19, ApoE4 knock-in (ε4 allele), and ApoE4/PS19. Sex was approximately balanced across genotypes, with each group consisting of roughly equal numbers of male and female mice (WT: 9 females, 8 males; PS19: 9 females, 7males; ApoE4: 8 females, 9 males; ApoE4/PS19: 10 females, 7 males). Some of the mice (64%) were used for longitudinal testing across different ages (3 and 7 months or 7 and 10 months) when possible. Approximately 15% of the animals either died of natural causes or were euthanized due to the development of hindlimb paralysis.

All mice were housed under standard 12-hour light/dark cycles with ad libitum access to food and water. No more than five adult mice were housed per cage. Animals were randomly assigned to experimental groups, and both sexes were included. All mice, regardless of genotype, were handled and monitored using the same protocols to ensure consistency across experimental conditions.

Auditory Brainstem Recordings (ABRs)

ABRs were recorded from mice across all genotypes: WT, PS19, ApoE4 knock-in (ε4 allele), and ApoE4/PS19 double transgenic mice at 3-4 months of age (n = 8-12 per group), 6-7 months of age (n = 12-18 per group), and 9-10 months of age (n = 12-18 per group). ABR recording procedures were performed as described in published literature (Chokr et al., 2022). Mice were anesthetized with ketamine (75 mg/kg i.m., KetaVed, VEDCO) and xylazine (15 mg/kg i.m., AnaSed, NADA #139-236) via intramuscular injection. Body temperature was maintained at 35°C using a far infrared warming pad (Kent Scientific, RT-0501), and sterile ocular lubricant (Retaine PM Ointment, SKU 777-3-75) was applied to the eyes. Three pin electrodes were inserted subcutaneously at the vertex, right cheek, and back near the right hind leg, corresponding to positive, negative, and ground electrodes, respectively. These electrodes were connected to Tucker-Davis Technologies (TDT) RA4PA 4-channel Medusa amplifier connected to a TDT RA16 Medusa Base Station (Figure 1B).

The ABRs were conducted in a sound-attenuating chamber (102 × 98 × 81 cm, Industrial Acoustics Company). Acoustic stimuli, including clicks and pure tones (4, 8, 12, 16, 24, and 32 kHz), were delivered to the left ear via an ear tube using a TDT MF1 Multi-Function Speaker. Stimuli were generated with TDT SigGen software (v4.4), played through a TDT RP2.1 enhanced real-time processor, and sound intensity was adjusted using a TDT PA5 programmable attenuator. Each stimulus was presented 500 times at a rate of 21 per second, and responses were amplified using a TDT SA1 stereo power amplifier, filtered, and recorded using BioSig software (v4.4) (Figure 1B).

Prior to ABR recordings, the acoustic system was calibrated under computer control from 5 kHz to 80 kHz in 5 Hz steps using a 0.5-inch condenser microphone (model 4134, Brüel & Kjær), ensuring accurate sound pressure level delivery across the frequency range tested. ABR responses were recorded over a 12-millisecond (ms) window for every 100 microseconds (μs) click or 3 ms tone burst, with 1.5 ms onset and 1.5 ms offset ramps. Sound levels were

decreased in 5 dB SPL steps from 80 to 10 dB SPL. Averaged waveforms at each intensity were used for threshold determination and ABR waveform analysis.

ABR Analysis

Assessment parameters for ABRs included hearing threshold, peak latency, interpeak latency, and peak amplitude. The threshold was defined as the lowest sound intensity at which the peak I level (uV) was ≥ 4 standard deviations above the noise level (Tanaka et al., 2023b). Raw ABR traces were normalized by MATLAB zero-phase filter (rloess, span = 2) and peaks were detected and labeled using Auditory Brainstem Response Waveform Analysis software (Burke et al., 2023) and manually checked by a blind observer. Peak latency was determined as the time from stimulus onset to the apex of the peak. Interpeak latency was calculated as the difference in peak latencies between peaks I-II, II-III, III-IV, and I-IV. Peak amplitude was determined as the change in microvolts (uV) between the apex of the peak and its subsequent trough.

Quantitative results are presented as mean values with error bars indicating the standard error of mean (SEM). All statistical analyses were performed using Python 3.10. Comparisons of ABR peak amplitudes, peak latencies, and interpeak latencies between genotypes (WT vs PS19 and WT vs ApoE4 control vs ApoE4/PS19 and PS19 vs ApoE4/PS19) and age groups (3 and 7 months) were made using Linear Mixed-Effects models (LME) with individual subjects included as a random factor, followed by pairwise post-hoc comparisons with False Discovery Rate (Benjamini-Hochberg) correction for multiple comparisons. Hearing thresholds were compared between groups using the nonparametric Mann-Whitney U test. Statistical significance was accepted at $p < 0.05$.

Behavioral Experiments

Prior to the experiments, mice were handled for 10 minutes each day for five days. Anxiety-like tests followed established protocols, with minor modifications. Statistical analysis of behavioral comparisons between genotypes (WT vs PS19 and WT vs ApoE4 control vs ApoE4/PS19) and/or age groups (3, 7, and 10 months) were performed using Prism Software (v9.3.1; GraphPad Software). Results were presented as mean ± SEM, with statistical significance accepted at $p < 0.05$.

Elevated Plus Maze (EPM)

The elevated plus-maze was placed 50 cm above the ground and had two open arms (25 cm × 5 cm) and two closed arms (25 cm × 5 cm × 15 cm) that were connected via a central platform (5 cm × 5 cm). The mice were placed onto the central platform facing the open arm and allowed to explore for 5 minutes. The number of entries and time spent in each arm and center zone were recorded by a blind observer. An entry was counted when all four paws of the animal entered a zone on the maze. The maze was cleaned with 70% ethanol, followed by distilled water, and then allowed to air-dry between each mouse (Figure 1C). Statistical analysis was performed using the Mann-Whitney t-test (WT vs PS19) and Kruskal-Wallis test, followed by Dunn's multiple comparisons (WT vs ApoE4 vs ApoE4/PS19) via Prism software.

Rotarod

For this test, the mice were placed onto a four-lane rod (1.25 inches in diameter), each lane measuring 4.5 inches in width, facing away from the experimenter (ROTOR-ROD™, San Diego Instrument). The rod was 18 inches above ground. On the training day, the mice were

allowed to freely explore and stand on the stationary rod for 2 minutes before the first trial. The rod was controlled by the rotor-rod™ software to accelerate steadily from 0 rpm to 15 rpm over 5 minutes. Foam mats (1 inch thickness) were placed on the ground below each lane to alleviate the impact upon falling. The mice were picked up and placed back on the rotating rod when they fell before the run was completed. The mice underwent four trials of 5 minutes each, with 15 minutes of rest between each trial. The rod was cleaned with 70% ethanol and distilled water between each mouse. On testing day, mice were placed onto the rod with an identical rotating and duration profile as training, except the fall latency (s) were automatically recorded by the rotor-rod™ software when the mice fell before the 5-minute run was completed. The testing phase included 5 trials, with a 20-minute rest between each trial. The rod was cleaned with 70% ethanol and distilled water between each mouse (Figure 1D). The average of the 5 trials for each animal was calculated and compiled for statistical analysis. Statistical analysis was performed using the Mann-Whitney test (WT vs PS19) and the Kruskal-Wallis test, followed by Dunn's multiple comparisons (WT vs ApoE4 vs ApoE4/PS19) via Prism software.

Results

Age-Dependent Changes in ABR Peak Amplitudes in PS19 and WT Mice

The ABR waveform reflects the electrical activity of neural populations along the ascending auditory pathway, with individual peaks corresponding to contributions from distinct but overlapping generators. Signals contributing to Peak I arise from the cochlea, spiral ganglion neurons, and the auditory (VIII) nerve. Peak II reflects activity within the cochlear nucleus, while Peak III receives contributions from cochlear nucleus cell populations and nuclei of the superior olivary complex (SOC) (Melcher and Kiang, 1996). Activity associated with the lateral lemniscus

gives rise to Peak IV (Møller et al., 1981) (Figure 1B). Representative ABR waveforms in response to tone stimuli are shown (Figure 2). Corresponding waveforms from click-evoked ABRs are provided in the supplementary materials (Figure S1-2). To assess the auditory pathway integrity across three age groups (3-4 months, 6-7 months, and 9-10 months) of PS19 and WT mice, hearing thresholds were quantified across all age groups. Peak amplitudes (μV), peak latencies (ms), and interpeak latencies (ms) were further analyzed in the 3-4 and 6-7 month cohorts, but not in the 9-10 month cohort, due to significant hearing loss observed during threshold screening in mice at this age. Hearing thresholds were compared between groups using the nonparametric Mann-Whitney U test. For all other ABR measurements, linear mixed-effects models were used to assess the main effect of age or genotype, with individual subjects included as a random factor and corrected by False Discovery Rate (FDR).

The aging effect on ABR was measured in age-matched PS19 and their WT mice using pure tone stimuli. Hearing thresholds in WT mice at 7 months were significantly elevated at 8, 16, and 24 kHz compared with 3-month-old controls ($p = 1.19 \times 10^{-2}$, $p = 1.36 \times 10^{-2}$, and $p = 1.58 \times 10^{-2}$, respectively), whereas 7-month-old PS19 mice exhibited higher thresholds at 24 kHz relative to their 3-month-old counterparts ($p = 4.87 \times 10^{-2}$), indicating modest, frequency-specific age-related differences in auditory sensitivity (Figure 3A, Table 1, Mann-Whitney U test). The peak IV-to-I amplitude ratio remained consistent between 3- and 7-month-old mice in both genotypes at all tested frequencies (WT: $p = 0.945$, $p = 0.945$, $p = 0.945$; PS19: $p = 0.692$, $p = 0.692$, $p = 0.692$ at 4, 12, and 24 kHz, respectively), with ratio values ≥1, indicating that the relative dynamics of brainstem responses are largely preserved with age (Figure 3B, LME with FDR correction).

Analysis of individual peak amplitudes revealed age-dependent changes across the auditory pathway. Peak amplitude was defined as the voltage difference (μV) between the apex of the peak and the immediately following trough (Figure 1B). Peak I amplitudes were

significantly reduced in 7-month-old mice compared with 3-month-old cohorts in both PS19 ($p = 7.44 \times 10^{-4}$, $p = 7.44 \times 10^{-4}$, $p = 7.43 \times 10^{-3}$ at 4, 12, and 24 kHz) and WT mice ($p = 7.12 \times 10^{-9}$, $p = 6.62 \times 10^{-9}$, $p = 5.59 \times 10^{-5}$ at 4, 12, and 24 kHz), consistent with a decline in peripheral auditory activity (Figure 3C, Table 1, LME with FDR correction). For Peak II, WT mice displayed significant age-related reduction across all tested frequencies ($p = 2.64 \times 10^{-7}$, $p = 4.64 \times 10^{-7}$, $p = 2.90 \times 10^{-3}$ at 4, 12, 24 kHz), whereas PS19 mice maintained comparable amplitudes between 3 and 7 months ($p = 0.339$, $p = 0.734$, $p = 0.339$ at 4, 12, 24 kHz), suggesting relative stability of early brainstem responses in the PS19 model (Figure 3D, Table 1, LME with FDR correction). Peak III amplitudes were comparable across ages in PS19 mice ($p = 0.686$, $p = 0.312$, $p = 0.891$ at 4, 12, 24 kHz), whereas WT mice exhibited significant age-related declines at 4 and 12 kHz ($p = 2.70 \times 10^{-6}$, $p = 2.20 \times 10^{-4}$, respectively), but not at 24 kHz ($p = 0.253$) (Figure 3E, Table 1, LME with FDR correction). Finally, Peak IV amplitudes were reduced significantly in 7-month-old mice in both genotypes across all frequencies (WT: $p = 8.59 \times 10^{-12}$, $p = 1.77 \times 10^{-10}$, $p = 3.88 \times 10^{-5}$; PS19: $p = 3.10 \times 10^{-14}$, $p = 2.43 \times 10^{-8}$, $p = 7.01 \times 10^{-8}$ at 4, 12, and 24 kHz, respectively) (Figure 3F, Table 1, LME with FDR correction).

<u>Presence of P301S mutation reduces peak amplitudes, peak latencies, and interpeak latencies</u>

To determine the effect of tauopathy development on the auditory pathway, we next examined the ABR peak amplitudes, peak latencies, and interpeak latencies across genotypes and ages. At 3 months, PS19 mice exhibited significantly elevated tone-evoked hearing thresholds at 8 kHz compared with age-matched WT mice ($p = 1.05 \times 10^{-3}$), indicating early deficits in auditory sensitivity (Figure 4A-B, Table 2, Mann-Whitney U test). By 7 months of age, hearing thresholds did not differ between genotypes across all tested frequencies ($p = 0.963$, $p = 0.852$, $p = 0.926$, $p = 0.563$, $p = 0.484$, $p = 0.317$ at 4, 8, 12, 16, 24, and 32 kHz, respectively)

for tone stimuli, suggesting partial normalization or compensatory changes with disease progression (Figure 4A-B, Table 2, Mann-Whitney U test).

Analysis of peak amplitudes revealed a reduction in neural response strength in PS19 mice (Figure 4). At 3 months of age, peak I amplitudes were significantly reduced in PS19 mice across the tested frequencies ($p = 1.05 \times 10^{-2}$, $p = 7.41 \times 10^{-3}$, $p = 4.75 \times 10^{-2}$ at 4, 12, and 24 kHz), reflecting impaired peripheral auditory nerve output. By 7 months, peak I amplitudes in PS19 mice became comparable with WT mice ($p = 0.38$, $p = 0.494$, $p = 0.635$ at 4, 12, and 24 kHz) (Figure 4C, Table 2 and S2, LME with FDR correction). Peak II amplitudes were reduced in PS19 mice at 3 months across all frequencies ($p = 3.01 \times 10^{-4}$, $p = 1.39 \times 10^{-3}$, $p = 3.24 \times 10^{-2}$ at 4, 12, 24 kHz) and increased at 4 ($p = 1.16 \times 10^{-2}$) and 12 kHz ($p = 1.16 \times 10^{-2}$) at 7 months relative to WT mice, suggesting frequency-specific alterations of cochlear nucleus activity with age (Figure 4D, Table 2 and S2, LME with FDR correction). Peak III amplitudes remained significantly reduced in PS19 mice at 3 months ($p = 9.80 \times 10^{-5}$, $p = 1.70 \times 10^{-4}$, $p = 1.70 \times 10^{-4}$ at 4, 12, and 24 kHz) and continued to be reduced at 12 ($p = 2.95 \times 10^{-3}$) and 24 kHz ($p = 9.24 \times 10^{-3}$) at 7 months, indicating persistent deficits within the superior olivary complex (Figure 4E, Table 2 and S2, LME with FDR correction). Peak IV amplitudes were reduced in PS19 mice at 3 months at 4 ($p = 3.78 \times 10^{-3}$) and 12 kHz ($p = 1.69 \times 10^{-3}$) and remained reduced at 7 months for 4 kHz ($p = 1.04 \times 10^{-4}$), illustrating changes in central brainstem auditory processing (Figure 4F, Table 2 and S2, LME with FDR correction).

To assess auditory signal transmission, peak latencies were examined across genotypes and ages. Peak latency is defined as the time (ms) between the initial auditory stimulus and a specific peak (Figure 1B). Peak I latencies were comparable between PS19 and WT mice at 3 ($p = 0.203$, $p = 0.539$, $p = 0.2.03$ at 4, 12, and 24 kHz) and 7 months across the tested frequencies ($p = 0.242$, $p = 0.509$, $p = 0.776$ at 4, 12, and 24 kHz), indicating preserved peripheral conduction timing (Figure 5A, Table 2 and S2, LME with FDR correction). In contrast, PS19 mice exhibited

significant delays in Peak II latencies at 3 months at 4 ($p = 6.88 \times 10^{-7}$) and 12 kHz ($p = 1.67 \times 10^{-2}$) that persisted to 7 months ($p = 3.77 \times 10^{-6}$, $p = 5.51 \times 10^{-3}$). At 24 kHz, peak II latencies remained comparable with WT mice at 3 months old ($p = 0.161$) and became delayed at 7 months ($p = 4.26 \times 10^{-2}$) (Figure 5B, Table 2 and S2, LME with FDR correction). Similarly, peak III latencies were delayed in PS19 mice at 3 months across tested frequencies ($p = 5.67 \times 10^{-4}$, $p = 1.38 \times 10^{-2}$, $p = 1.69 \times 10^{-2}$ at 4, 12 and 24 kHz), with persistent delays at 7 months at 4 ($p = 3.76 \times 10^{-6}$) and 12 kHz ($p = 6.86 \times 10^{-7}$) (Figure 5C, Table 2 and S2, LME with FDR correction). Peak IV latencies were also delayed in PS19 mice at 3 months at 4 ($p = 1.80 \times 10^{-24}$), 12 kHz ($p = 1.85 \times 10^{-5}$) and 24 kHz ($p = 4.43 \times 10^{-2}$) and remained delayed at 7 months ($p = 9.02 \times 10^{-6}$, $p = 6.81 \times 10^{-5}$) for 4 and 12 kHz, indicating sustained slowing of neural conduction within more central auditory pathways (Figure 5D, Table 2 and S2, LME with FDR correction).

Interpeak latency analysis further revealed impaired neural transmission between successive centers along the auditory brainstem pathway in PS19 mice. Interpeak latency is defined as the duration (ms) between two sequential peaks (Figure 1B). At 3 months, interpeak I-II latencies were significantly delayed in PS19 mice at 4 ($p = 8.45 \times 10^{-9}$) and 12 kHz ($p = 9.02 \times 10^{-3}$), and remained delayed at 4 ($p = 8.45 \times 10^{-3}$), 12 ($p = 2.92 \times 10^{-2}$), and 24 kHz ($p = 2.92 \times 10^{-2}$) at 7 months (Figure 6A, Table 2 and S2, LME with FDR correction). Interpeak II-III latencies were comparable between genotypes at 3 months ($p = 0.516$, $p = 0.629$, $p = 0.516$ at 4, 12, and 24 kHz) but delayed significantly in PS19 mice at 7 months at 4 ($p = 2.73 \times 10^{-3}$) and 12 kHz ($p = 2.73 \times 10^{-3}$), suggesting progressive dysfunction in brainstem relay timing (Figure 6B, Table 2 and S2, LME with FDR correction). Interpeak III-IV latencies were comparable with WT mice at 3 months ($p = 9.72 \times 10^{-2}$, $p = 0.194$, $p = 0.663$ at 4, 12, and 24 kHz) and at 7 months ($p = 0.981$, $p = 0.981$, $p = 0.522$, respectively) (Figure 6C, Table 2 and S2, LME with FDR correction). Finally, the composite I-IV interpeak latency was significantly delayed in PS19 mice at both 3 and 7 months at 4 ($p = 2.76 \times 10^{-35}$ at 3 months, $p = 1.96 \times 10^{-5}$ at 7 months) and 12 kHz ($p = 6.72 \times 10^{-}$

^{9} at 3 months, $p = 1.32 \times 10^{-5}$ at 7 months), while remaining comparable between genotypes at 24 kHz ($p = 8.19 \times 10^{-2}$ at 3 months, $p = 0.277$ at 7 months) (Figure 6D, Table 2 and S2, LME with FDR correction).

To further characterize auditory dysfunction in PS19 mice, click-evoked ABR responses were examined, which assess broadband cochlear activation rather than frequency-specific processing. Click-evoked thresholds were elevated relative to WT at 7 months ($p = 3.26 \times 10^{-2}$) (Figure S1, Table S1, Mann-Whitney U test), and peak and interpeak latencies were shortened at 3 months, with effects largely resolving by 7 months. Peak amplitudes remained mostly comparable with WT across both ages (Figure S1, Table S1, LME with FDR correction).

Age-Dependent Changes in ABR Peak Amplitudes in ApoE4/PS19 and ApoE4 Mice

To examine auditory function in the context of ApoE4 and tauopathy, we measured ABR thresholds and peak amplitudes in 3- and 7-month-old ApoE4/PS19 and ApoE4 control mice. Hearing thresholds differed with age in both genotypes. At 7 months, ApoE4 controls exhibited elevated hearing thresholds at 4 ($p = 3.75 \times 10^{-3}$), 8 ($p = 9.65 \times 10^{-3}$), 12 ($p = 6.85 \times 10^{-3}$), 16 ($p = 1.23 \times 10^{-3}$), and 24 kHz ($p = 2.62 \times 10^{-3}$) compared to their 3-month-old counterparts, while 7-month-old ApoE4/PS19 mice showed elevated hearing thresholds at 8 ($p = 3.76 \times 10^{-3}$), 16 ($p = 5.26 \times 10^{-3}$), 24 ($p = 5.87 \times 10^{-4}$), and 32 kHz ($p = 7.64 \times 10^{-3}$), relative to 3-month-old cohorts (Figure 7A, Table 1, Mann-Whitney U test). These findings indicate frequency-specific age-related differences in auditory sensitivity. The peak IV-to-I amplitude ratio remained consistent between 3- and 7-month-old mice in ApoE4 controls at the shown frequencies ($p = 0.806$, $p = 0.992$, $p = 0.105$ at 4, 12, and 24 kHz, respectively), with ratio values <1, indicating reduced central amplification relative to peripheral inputs. The peak IV-to-I amplitude ratio remained consistent

between 3- and 7-month-old mice in ApoE4/PS19 controls at 4 ($p = 0.480$), 12 kHz ($p = 0.990$), and at 24 kHz ($p = 0.884$) (Figure 7B, LME with FDR correction).

Analysis of individual peaks revealed additional age-dependent patterns. Peak I amplitudes were reduced in 7-month-old mice compared with 3-month-old cohorts in both ApoE4/PS19 ($p = 7.32 \times 10^{-20}$, $p = 7.32 \times 10^{-20}$, $p = 2.20 \times 10^{-7}$ at 4, 12, and 24 kHz) and ApoE4 controls ($p = 1.26 \times 10^{-5}$, $p = 1.00 \times 10^{-12}$, $p = 1.26 \times 10^{-5}$ at 4, 12, and 24 kHz), consistent with reduced peripheral auditory nerve activity with increasing age (Figure 7C, Table 1, LME with FDR correction). Peak II amplitudes in ApoE4/PS19 mice remained comparable with age at 4 ($p = 0.848$) and 12 ($p = 0.627$) and reduced at 24 kHz ($p = 5.93 \times 10^{-6}$), suggesting selective central auditory alterations. Peak II amplitudes also remained comparable at 4 ($p = 0.317$), 12 ($p = 0.202$), and at 24 kHz ($p = 5.63 \times 10^{-2}$) in ApoE4 controls (Figure 7D, Table 1, LME with FDR correction). Peak III amplitudes were reduced with age across all tested frequencies in both genotypes (ApoE4/PS19: $p = 1.64 \times 10^{-17}$, $p = 2.10 \times 10^{-19}$, $p = 3.82 \times 10^{-14}$; ApoE4: $p = 1.65 \times 10^{-13}$, $p = 1.87 \times 10^{-11}$, $p = 1.04 \times 10^{-24}$ at 4, 12, and 24 kHz, respectively) (Figure 7E, Table 1, LME with FDR correction). Peak IV amplitudes remained comparable between age groups at all frequencies (ApoE4/PS19: $p = 0.533$, $p = 0.533$, $p = 0.425$; ApoE4: $p = 0.442$, $p = 0.234$, $p = 0.565$ at 4, 12, and 24 kHz, respectively) (Figure 7F, Table 1, LME with FDR correction).

<u>ApoE4 allele contributes to auditory dysfunction</u>

To examine the independent and combined effects of ApoE4 and tau pathology on auditory function, we compared ABR responses in WT, PS19, and ApoE4/PS19 mice at 3 and 7 months of age (Figure 8). At 3 months, ApoE4/PS19 mice exhibited significantly elevated hearing thresholds at 12 ($p = 3.60 \times 10^{-2}$) and 16 kHz ($p = 1.10 \times 10^{-2}$) compared with WT mice, indicating early deficits in auditory sensitivity. At the same age, ApoE4/PS19 mice showed a reduction at 8

384 ($p = 2.43 \times 10^{-2}$) and an elevation in hearing threshold at 16 kHz ($p = 1.65 \times 10^{-2}$) compared to the
385 PS19 mice. By 7 months of age, ApoE4/PS19 mice showed elevated hearing thresholds at 12 (p
386 $= 3.62 \times 10^{-2}$), 16 ($p = 1.50 \times 10^{-2}$), and 24 kHz ($p = 4.18 \times 10^{-2}$) compared to PS19 mice, while
387 being comparable with WT mice across all tested frequencies ($p = 0.568$, $p = 0.646$, $p = 0.239$, p
388 $= 0.229$, $p = 0.573$, $p = 0.369$ at 4, 8, 12, 16, 24, and 32 kHz, respectively) for tone stimuli,
389 suggesting partial normalization or compensatory changes with disease progression (Figure 8A-
390 B, Table 3-4 and S3, Mann-Whitney U test).

391 Analysis of peak amplitudes, latencies, and interpeak latencies revealed age- and
392 frequency-dependent alterations in neural response strength. Peak I amplitudes showed
393 significant reductions in both ApoE4/PS19 mice compared to PS19 and WT mice across all
394 frequencies at both 3 (PS19: $p = 1.58 \times 10^{-46}$, $p = 1.75 \times 10^{-43}$, $p = 2.15 \times 10^{-20}$; WT: $p = 1.31 \times 10^{-32}$, $p = 1.29 \times 10^{-29}$, $p = 2.24 \times 10^{-16}$) and 7 months (PS19: $p = 1.82 \times 10^{-78}$, $p = 6.72 \times 10^{-47}$, $p = 2.01 \times 10^{-20}$; WT: $= 6.92 \times 10^{-50}$, $p = 1.54 \times 10^{-38}$, $p = 6.58 \times 10^{-16}$ for 4, 12 and 24 kHz, respectively)
397 (Figure 8C, Table 3-4 and S3, LME with FDR correction). Peak II amplitudes were similarly
398 reduced in ApoE4/PS19 compared to PS19 and WT mice. At 3 months, ApoE4/PS19 showed
399 significant reductions across frequencies (PS19: $p = 8.31 \times 10^{-13}$, $p = 2.39 \times 10^{-20}$, $p = 2.23 \times 10^{-3}$; WT: $p = 1.11 \times 10^{-18}$, $p = 3.30 \times 10^{-34}$, $p = 1.15 \times 10^{-160}$). At 7 months, ApoE4/PS19 remained
401 significantly reduced (PS19: $p = 2.59 \times 10^{-16}$, $p = 1.96 \times 10^{-14}$, $p = 2.61 \times 10^{-4}$; WT: $p = 2.59 \times 10^{-9}$, $p = 1.79 \times 10^{-9}$, $p = 8.72 \times 10^{-4}$) (Figure 8D, Table 3-4 and S3, LME with FDR correction). Peak
403 III amplitudes were comparable between ApoE4/PS19 and WT mice at 3 months at 4 ($p = 0.734$)
404 and 12 kHz ($p = 0.935$) and increased at 24 kHz ($p = 1.01 \times 10^{-2}$). Compared to PS19 at the same
405 age, ApoE4/PS19 amplitudes were increased across tested frequencies ($p = 1.49 \times 10^{-4}$, $p = 1.58 \times 10^{-6}$, $p = 3.89 \times 10^{-23}$). At 7 months, modest reductions occurred in ApoE4/PS19 mice at 4 and
407 12 kHz ($p = 3.51 \times 10^{-4}$, $p = 2.22 \times 10^{-4}$), while 24 kHz responses remained comparable ($p = 5.86 \times 10^{-2}$) to WT mice. ApoE4/PS19 peak amplitudes were reduced at 4 kHz ($p = 9.04 \times 10^{-3}$) and

409 became comparable at higher frequencies ($p = 0.497$, $p = 0.314$ for 12 and 24 kHz) relative to
410 PS19 mice (Figure 8E, Table 3-4 and S3, LME with FDR correction). Peak IV amplitudes showed
411 consistent reductions in ApoE4/PS19 compared to PS19 and WT mice across all frequencies and
412 ages (3 months - PS19: $p = 2.16 \times 10^{-103}$, $p = 7.62 \times 10^{-107}$, $p = 3.62 \times 10^{-66}$; WT: $p = 3.42 \times 10^{-86}$,
413 $p = 8.43 \times 10^{-102}$, $p = 2.67 \times 10^{-45}$ and 7 months - PS19: $p = 4.94 \times 10^{-97}$, $p = 1.07 \times 10^{-79}$, $p = 4.39$
414 $\times 10^{-25}$; WT: $p = 1.06 \times 10^{-86}$, $p = 1.60 \times 10^{-69}$, $p = 3.34 \times 10^{-47}$ for 4, 12 and 24 kHz, respectively)
415 (Figure 8F, Table 3-4 and S3, LME with FDR correction).

416 Peak latency analysis revealed consistent alterations in neural response timing in both
417 ApoE4/PS19 mice compared to PS19 and WT mice, characterized by delayed latencies at peak
418 I and shortened latencies at peaks II, III, and IV (Figure 9). Peak I latencies were significantly
419 delayed in ApoE4/PS19 mice compared to PS19 and WT mice. At 3 months, ApoE4/PS19 mice
420 showed comparable latencies to PS19 and WT mice at 4 kHz ($p = 0.207$, $p = 0.812$) and at 12 (p
421 $= 0.346$, $p = 0.114$), with pronounced delays emerging at 24 kHz ($p = 4.73 \times 10^{-19}$, $p = 1.23 \times 10^{-}$
422 ^{14} for PS19 and WT, respectively). By 7 months, delayed latencies were evident across all tested
423 frequencies in comparison to PS19 ($p = 1.17 \times 10^{-13}$, $p = 7.58 \times 10^{-16}$, $p = 4.57 \times 10^{-11}$) and WT
424 mice ($p = 1.19 \times 10^{-19}$, $p = 5.44 \times 10^{-19}$, $p = 6.60 \times 10^{-9}$ at 4, 12 and 24 kHz, respectively) (Figure
425 9A, Table 3-4 and S3, LME with FDR correction). Peak II latencies showed ApoE4/PS19
426 exhibiting significantly shortened latencies compared to PS19 and WT mice. At 3 months, the
427 effects were pronounced across tested frequencies (PS19: $p = 1.80 \times 10^{-144}$, $p = 9.14 \times 10^{-79}$, $p =$
428 1.11×10^{-10}; WT: $p = 4.13 \times 10^{-88}$, $p = 1.27 \times 10^{-58}$, $p = 3.26 \times 10^{-4}$ for 4, 12, and 24 kHz,
429 respectively). At 7 months, shortened latencies persisted across 4 ($p = 1.29 \times 10^{-7}$), and 12 kHz
430 ($p = 4.00 \times 10^{-14}$) relative to WT, and across tested frequencies relative to PS19 mice ($p = 5.99 \times$
431 10^{-17}, $p = 7.56 \times 10^{-21}$, $p = 3.31 \times 10^{-2}$ for 4, 12, and 24 kHz, respectively) (Figure 9B, Table 3-4
432 and S3, LME with FDR correction). Peak III latencies were shortened in ApoE4/PS19 mice
433 compared to PS19 and WT mice across tested frequencies. At 3 months, ApoE4/PS19 showed

shortened latencies at all frequencies (PS19: $p = 2.34 \times 10^{-118}$, $p = 7.39 \times 10^{-144}$, $p = 3.52 \times 10^{-61}$; WT: $p = 5.07 \times 10^{-52}$, $p = 2.53 \times 10^{-73}$, $p = 5.96 \times 10^{-26}$ for 4, 12, and 24 kHz). At 7 months, latency shortening persisted in comparison to both PS19 ($p = 9.17 \times 10^{-34}$, $p = 6.58 \times 10^{-49}$, $p = 6.01 \times 10^{-7}$) and WT ($p = 1.84 \times 10^{-15}$, $p = 4.35 \times 10^{-37}$, $p = 2.54 \times 10^{-4}$ at 4, 12, and 24 kHz) mice (Figure 9C, Table 3-4 and S3, LME with FDR correction). Peak IV latencies showed consistent shortening in ApoE4/PS19 compared to PS19 and WT mice across all frequencies and ages (3 months - PS19: $p = 1.00 \times 10^{-308}$, $p = 1.00 \times 10^{-308}$, $p = 6.63 \times 10^{-109}$; WT: $p = 4.36 \times 10^{-254}$, $p = 2.46 \times 10^{-225}$, $p = 2.05 \times 10^{-76}$ and 7 months - PS19: $p = 1.69 \times 10^{-76}$, $p = 1.12 \times 10^{-87}$, $p = 4.31 \times 10^{-15}$; WT: $p = 8.86 \times 10^{-45}$, $p = 3.39 \times 10^{-78}$, $p = 3.35 \times 10^{-10}$ for 4, 12, and 24 kHz, respectively) (Figure 9D, Table 3-4 and S3, LME with FDR correction).

Interpeak latencies reveal altered neural transmission timing in ApoE4/PS19 mice (Figure 10). Peak I-II interpeak latencies were significantly shortened in ApoE4/PS19 mice compared to PS19 and WT mice. At 3 months, interpeak latencies are shortened across frequencies (PS19: $p = 5.30 \times 10^{-153}$, $p = 7.15 \times 10^{-110}$, $p = 3.24 \times 10^{-33}$; WT: $p = 2.03 \times 10^{-113}$, $p = 2.43 \times 10^{-115}$, $p = 2.11 \times 10^{-29}$ for 4, 12, and 24 kHz). By 7 months, shortened I-II intervals persisted in ApoE4/PS19 across all tested frequencies (PS19: $p = 1.01 \times 10^{-69}$, $p = 9.82 \times 10^{-73}$, $p = 1.12 \times 10^{-30}$; WT: $p = 1.91 \times 10^{-51}$, $p = 7.80 \times 10^{-65}$, $p = 1.71 \times 10^{-15}$ for 4, 12, and 24 kHz, respectively) (Figure 10A, Table 3-4 and S3, LME with FDR correction). II-III interpeak latencies were shortened in ApoE4/PS19 mice compared to PS19 and WT mice. At 3 months, ApoE4/PS19 mice exhibited shortened II-III intervals across frequencies (PS19: $p = 1.37 \times 10^{-3}$, $p = 1.00 \times 10^{-33}$, $p = 3.64 \times 10^{-15}$; WT: $p = 1.03 \times 10^{-2}$, $p = 1.04 \times 10^{-8}$, $p = 1.40 \times 10^{-10}$). At 7 months, shortened II-III intervals continued for tested frequencies (PS19: $p = 7.80 \times 10^{-14}$, $p = 2.56 \times 10^{-24}$, $p = 2.59 \times 10^{-8}$; WT: $p = 3.10 \times 10^{-7}$, $p = 1.53 \times 10^{-14}$, $p = 5.30 \times 10^{-11}$ for 4, 12, and 24 kHz, respectively) (Figure 10B, Table 3-4 and S3, LME with FDR correction). III-IV interpeak latencies were shortened in ApoE4/PS19 mice compared to PS19 and WT mice across tested conditions. At both ages,

ApoE4/PS19 nice showed significantly shortened III-IV intervals (3 months - PS19: $p = 5.70 \times 10^{-31}$, $p = 2.47 \times 10^{-45}$, $p = 7.32 \times 10^{-41}$; WT: $p = 3.00 \times 10^{-23}$, $p = 1.44 \times 10^{-36}$, $p = 3.27 \times 10^{-26}$ and 7 months - PS19: $p = 1.12 \times 10^{-40}$, $p = 1.01 \times 10^{-36}$, $p = 1.02 \times 10^{-9}$; WT: $p = 6.72 \times 10^{-38}$, $p = 8.61 \times 10^{-40}$, $p = 1.08 \times 10^{-15}$ for 4, 12, and 24 kHz, respectively) (Figure 10C, Table 3-4 and S3, LME with FDR correction). I-IV interpeak latencies were shortened in ApoE4/PS19 mice (3 months: $p = 1.72 \times 10^{-303}$, $p = 1.00 \times 10^{-308}$, $p = 1.99 \times 10^{-230}$; 7 months: $p = 1.43 \times 10^{-74}$, $p = 5.15 \times 10^{-180}$, $p = 2.09 \times 10^{-21}$) and ApoE4/PS19 mice (3 months - PS19: $p = 1.00 \times 10^{-308}$, $p = 1.00 \times 10^{-308}$, $p = 1.00 \times 10^{-308}$; WT: $p = 6.02 \times 10^{-302}$, $p = 1.00 \times 10^{-308}$, $p = 4.02 \times 10^{-130}$; 7 months - PS19: $p = 6.80 \times 10^{-139}$, $p = 7.99 \times 10^{-160}$, $p = 3.63 \times 10^{-66}$; WT: $p = 2.13 \times 10^{-104}$, $p = 1.46 \times 10^{-152}$, $p = 1.56 \times 10^{-44}$ for 4, 12, and 24 kHz, respectively) compared to PS19 and WT mice across all frequencies and ages (Figure 10D, Table 3-4 and S3, LME with FDR correction).

A direct comparison of ApoE4 and ApoE4/PS19 mice showed minimal significant differences across both tone- and click-evoked ABR measures, indicating that PS19-mediated tau pathology does not meaningfully worsen the auditory impairments associated with ApoE4 alone (Figure S2-5, Table S3-4, LME with FDR correction). Comparisons between ApoE4 and WT mice followed a similar pattern, with most significant differences consistent with those seen in ApoE4/PS19 versus WT. This further supports ApoE4 as the primary contributor to auditory dysfunction in these mice, with tau pathology playing a limited additional role (Figure S6-8, Table S2-4, LME with FDR correction).

Preserved anxiety-like behavior and motor functions in PS19 mice

To determine whether sensory dysfunction, such as hearing impairments, precedes behavioral impairments, we examined anxiety-like behavior and motor performance in PS19 mice at 3, 7, and 10 months of age. In the elevated plus maze (EPM), PS19 spent comparable time in

the open, closed, and center arms at 3 and 7 months of age relative to WT mice (3 months: $p = 9.14 \times 10^{-2}$, $p = 0.317$, $p = 0.456$; 7 months: $p = 0.369$, $p = 0.865$, $p = 0.635$) while spending more time in open-arms at 10 months ($p = 0.195$, $p = 0.859$, $p = 1.40 \times 10^{-2}$) for open, center and closed arms, respectively), indicating reduced anxiety-like behaviors at later age (Figure 11A, Table 5, Mann Whitney test).

Rotarod testing revealed no significant differences in motor performance between PS19 mice and WT mice at either 3, 7, or 10 months of age. Latencies to fall were comparable across genotypes and ages (3 months: $p = 0.878$; 7 months: $p > 0.999$; 10 months: $p = 0.599$), indicating preserved motor coordination and endurance in PS19 mice during these periods (Figure 11B, Table 5, Mann-Whitney t-test).

Age-Dependent Changes in Anxiety-Like and Motor Function in ApoE4/PS19 Mice

Behavioral analysis was conducted for ApoE4/PS19 mice to assess their anxiety-like behavior and motor performance at 3, 7, and 10 months of age. In EPM test, ApoE4/PS19 mice showed age-dependent differences in exploratory behavior relative to both ApoE4 and WT mice. At 3 months, ApoE4/PS19 mice spent less time in the closed arms ($p = 3.20 \times 10^{-3}$) and more time in the center zone ($p = 3.30 \times 10^{-3}$) relative to ApoE4 controls, while remaining comparable in open arm time ($p > 0.999$). Compared to WT, ApoE4/PS19 mice spent comparable time in closed ($p = 7.86 \times 10^{-2}$) and open ($p > 0.999$) arms, but more time in the center zone ($p = 1.73 \times 10^{-2}$), indicating that the combination of ApoE4 and tau pathology selectively increases center zone occupancy relative to ApoE4 alone at this age. At 7 months, time spent in closed arms, center, and open arms was comparable across all three genotypes (WT vs. ApoE4: $p = 0.183$, $p = 0.519$, $p = 0.364$; WT vs. ApoE4/PS19: $p > 0.999$, $p > 0.999$, $p = 0.917$; ApoE4 vs. ApoE4/PS19: $p = 0.599$, $p = 0.952$, $p > 0.999$, for closed, center and open arms, respectively). At 10 months,

ApoE4/PS19 mice spent less time in the closed arms ($p = 3.33 \times 10^{-2}$) and more time in the center zone ($p = 2.20 \times 10^{-3}$) compared to ApoE4 mice. WT mice also showed elevated center zone time relative to ApoE4 controls ($p = 4.00 \times 10^{-3}$), suggesting diverging trajectories of anxiety-like behavior in WT, ApoE4, and ApoE4/PS19 mouse models with increasing age (Figure 11C, Table 5, Kruskal-Wallis, followed by Dunn's multiple comparisons).

Rotarod testing reveals age-dependent differences in motor performance. At 3 months, latency to fall was comparable across all three genotypes (WT vs. ApoE4: $p = 0.352$; WT vs. ApoE4/PS19: $p = 0.986$; ApoE4 vs. ApoE4/PS19: $p > 0.999$). At 7 months, ApoE4/PS19 mice exhibited significantly greater latency to fall compared to both WT ($p < 1.00 \times 10^{-4}$) and ApoE4 controls ($p = 3.20 \times 10^{-3}$). This pattern persisted for 10 months, with ApoE4/PS19 mice again showing greater latency to fall relative to both WT ($p = 4.00 \times 10^{-4}$) and ApoE4 controls ($p = 1.06 \times 10^{-2}$) (Figure 11D, Table 5, Kruskal-Wallis followed by Dunn's multiple comparisons).

Discussion

This study shows that auditory pathway dysfunction may represent an early and sensitive development of tau pathology in the PS19 mouse model, emerging before detectable motor and anxiety-like behavioral changes. Through comprehensive assessment of neural transmission along the ascending auditory brainstem pathway, our data reveal tauopathy association with progressive, frequency-specific alterations in auditory processing that precede behavioral disease phenotypes. The interaction between P301S mutation and the ApoE4 risk allele produces distinct temporal patterns of auditory impairments, with implications for understanding the progression of Alzheimer's disease and related tauopathies.

Auditory pathway dysfunction precedes behavioral impairment in PS19 mice

A central finding of this study is that auditory pathway dysfunction precedes cognitive impairment and neuronal loss in PS19 mice, coinciding with the earliest stages of tauopathy. At 3 months of age, before neurofibrillary tangle formation and cognitive deficits (Yoshiyama et al., 2007), PS19 mice showed reduced neural response amplitudes across multiple brainstem auditory nuclei and slowed signal transmission throughout the auditory pathway, in animals that showed no detectable changes in anxiety-like behavior or motor coordination. Reduced peripheral auditory nerve activity (Peak I) coupled with elevated hearing thresholds at 8 kHz suggests that tau pathology affects peripheral sensory structures at disease onset in P301S models, consistent with tau expression in peripheral spiral ganglion neurons (Wang and Wu, 2021). The widespread nature of early auditory deficits, extending from the peripheral auditory nerve through multiple brainstem auditory nuclei, suggests that the auditory system is among the earliest vulnerable neural networks in tauopathy.

Auditory pathway dysfunction reveals stage-specific patterns in PS19 mice

The temporal progression of auditory deficits across brainstem auditory centers reveals a complex pattern of regional dysfunction and compensation. While age-related amplitude declines occurred across all genotypes, PS19 mice exhibited a distinct trajectory: Peak I declined similarly to controls, Peak II remained stable, and Peak III showed relative preservation, suggesting tau pathology triggers region-specific compensatory responses that alter normal aging trajectories.

Peripheral auditory nerve function showed initial compromise, followed by apparent normalization by 7 months, potentially reflecting compensatory mechanisms in surviving neurons (Schrode et al., 2018; Johannesen et al., 2021). In contrast, central auditory structures showed persistent or progressive dysfunction: superior olivary complex activity (Peak III) remained significantly impaired at both ages, and lateral lemniscus function (Peak IV) showed

554 progressive deterioration. Because the Wave IV/Wave I amplitude ratio was not significantly
555 altered, these reductions may partly reflect reduced peripheral drive rather than independent
556 central pathology.

557 Particularly striking was the cochlear nucleus (Peak II): initial reduction at 3 months
558 followed by elevation above control levels at 7 months. This reversal likely reflects
559 compensatory hyperactivity within the cochlear nucleus, possibly involving increased neuronal
560 excitability or altered inhibitory-excitatory balance as the structure attempts to compensate for
561 diminished peripheral input (Auerbach et al., 2014; Schrode et al., 2018). Persistent slowing of
562 neural transmission reflected in prolonged I-IV interpeak latencies suggests that temporal
563 processing deficits are a core feature of auditory dysfunction in tauopathy, likely arising from
564 axonal dysfunction, compromised myelination, or synaptic deficits associated with tau
565 accumulation. (Kneynsberg et al., 2017; Tracy and Gan, 2018).

566 The frequency-specific nature of these deficits, with relative preservation at 24 kHz,
567 indicates differential impairment within the tonotopic organization of auditory structures
568 susceptible to tauopathy. This selectivity may reflect regional differences in tau expression,
569 metabolic demands, or cellular composition that confer protection or risk to specific neuronal
570 populations within the auditory pathway. Click and tone ABRs reflect distinct cochlear regions
571 and synchrony demands, explaining their opposing latency patterns in PS19 mice (Melcher and
572 Kiang, 1996).

573 ApoE4 allele alters the trajectory of auditory pathway dysfunction

574 The human ApoE4 allele produced a broad and stable pattern of auditory dysfunction
575 that differed from tau-related decline in PS19 mice. Both ApoE4 and ApoE4/PS19 mice showed
576 elevated hearing thresholds at 3 months relative to WT mice, with partial normalization by 7

months, suggesting early peripheral sensitivity deficits that are partially compensated over time. Amplitude abnormalities were far more persistent: Peaks I, II, and IV remained significantly reduced at both ages, indicating sustained response strength deficits that involve mechanisms distinct from those underlying threshold sensitivity.

Our analysis revealed directionally opposite changes in peak latency: Peaks II, III, and IV latencies were uniformly shortened relative to WT mice, while Peak I latency was delayed, a pattern that held across frequencies and ages that became more pronounced by 7 months. The delayed Peak I latency likely reflects compromised peripheral auditory nerve conduction via ApoE4-mediated disruption of lipid homeostasis and demyelination of spiral ganglion neuron axons (Chen et al., 2025a). In contrast, shortened central latencies at Peaks II-IV may reflect ApoE4-driven reductions in inhibitory tone within auditory brainstem stations where ApoE4 is associated with loss of GABAergic interneuron function and reduced inhibitory-excitatory balance (Nuriel et al., 2017; Najm et al., 2019), which would enhance neural synchrony and accelerate apparent signal propagation through central auditory structures. Further analysis revealed shortened interpeak latencies across all intervals consistent with this disinhibition model. Together, this opposing peripheral-central pattern points to ApoE4-mediated disruption of myelination and inhibitory circuit integrity as primary drivers of auditory brainstem reorganization, rather than uniform deterioration.

Cochlear nucleus function differed remarkably between ApoE4 and PS19 backgrounds. While PS19 mice showed elevated Peak II amplitudes at 7 months, consistent with compensatory hyperexcitability, ApoE4/PS19 mice exhibited reduced amplitudes. ApoE4 mice alone showed partial recovery of Peak II amplitudes toward WT levels by 7 months, whereas ApoE4/PS19 mice remained significantly reduced, representing one of the few points of divergence between the two mutant genotypes. This suggests that while ApoE4 alone may permit partial compensatory normalization of cochlear nucleus output, the addition of PS19-

mediated tau pathology limits this recovery. Whether this reflects tau-driven suppression of compensatory plasticity or ApoE4-tau interactions at the level of cochlear nucleus circuitry requires further investigation.

This genotype-dependent difference in auditory response may reflect ApoE4-mediated alterations in tau propagation, neuroinflammatory responses, or compensatory plasticity (Shi et al., 2017; Friedberg et al., 2020; Koutsodendris et al., 2023). Despite these differences, direct comparisons between ApoE4 and ApoE4/PS19 mice revealed minimal differences across the majority of ABR measures, indicating that PS19-mediated tau pathology does not substantially worsen the auditory impairments already conferred by ApoE4 alone. This raises the possibility that ApoE4 engages pathological mechanisms that occlude further deterioration from tau accumulation, perhaps through shared downstream effects on synaptic or myelination (Koffie et al., 2012; Blanchard et al., 2022) or that ApoE4 alters the regional distribution of tau in ways that mask additive effects at the time points examined.

The absence of anxiety-like or motor impairments in PS19 mice at 3, 7, and 10 months despite robust auditory dysfunction supports sequential neural system vulnerability, reflecting circuit-specific thresholds for dysfunction. In ApoE4/PS19 mice, the behavioral profile was distinct from either parental genotype alone. Reduced closed-arm time and elevated center zone occupancy at 3 and 10 months, and altered rotarod performance from 7 months onward, suggest that ApoE4 and tau pathology interact to produce a qualitatively different phenotype rather than additive impairment. Tau pathology may attenuate the heightened anxiety characteristic of ApoE4 mice (Siegel et al., 2010), consistent with reports of hyperactivity and reduced anxiety in PS19 mice with advancing tau burden (Ahmad et al., 2021). Elevated center zone time may reflect behavioral conflicts rather than reduced anxiety, aligning with the classic approach-avoidance framework in rodents (Salum, et al., 2003, Montgomery, 1995), since entering open arms requires overriding competing avoidance drives that EPM data alone cannot

resolve. The paradoxical improvement in rotarod performance may reflect similar disinhibition, with ApoE4/PS19 mice engaging the task more persistently than anxious ApoE4 counterparts. ApoE4 also exacerbates tau pathology in P301S mice (Shi et al., 2017), raising the possibility that ApoE4/PS19 mice carry greater tau burden than PS19 alone, potentially disrupting anxiety circuitry more extensively.

Study limitations and implications

All experimental mice were maintained on a B6C3F1/J hybrid background, heterozygous at the Cdh23 locus; moderately elevated baseline ABR thresholds in WT mice are consistent with this background and are shared equally across genotypes. Our behavioral assays could be expanded; auditory-dependent cognitive tasks may reveal more subtle impairments. The mechanisms underlying the selective auditory pathway vulnerability remain unclear, highlighting the need to investigate molecular features that confer early sensitivity to tau pathology.

Our findings support growing evidence that auditory dysfunction represents an early, clinically relevant feature of tauopathies and AD/ADRD, given ApoE4's role as a major genetic risk factor. Hearing impairment is a major modifiable dementia risk factor (Su et al., 2017; Azeem et al., 2023), yet mechanisms linking auditory dysfunction to cognitive decline remain unclear. The auditory pathway's demands for high temporal precision and extensive axonal projections may render it susceptible to tauopathy. ApoE4's modulation of auditory phenotypes reveals that genetic context shapes not only disease risk but also the trajectory of neural system involvement. The distinct pattern of deficits observed in ApoE4/PS19 mouse models suggests that dynamic patterns of dysfunction and compensation, and potential reemergence may matter

more to cognitive outcomes than severity at single time point, a temporal dimension accessible only through longitudinal assessment that may represent a critical therapeutic window.

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Figure Legends

Figure 1. Experimental overview and behavioral paradigms. **(A)** Schematic of the experimental timeline illustrating the sequence of experiments performed. **(B)** Diagram of the Auditory Brainstem Response (ABR) experimental setup. Yellow, red, and green needles indicate electrode placement on the animal. A representative ABR waveform is shown with the measured parameters indicated. **(C)** Schematic of the Elevated Plus Maze (EPM) apparatus, consisting of two open arms and two closed arms elevated 50 cm above the ground, connected by a central platform. **(D)** Schematic of the rotarod test used to assess motor coordination and balance. Latency to fall was recorded across five trials, with a 20-minute rest period between trials.

Figure 2. Auditory brainstem response analysis and waveforms in PS19 and PS19/ApoE4 mouse models across ages. **(A)** Representative workflow for ABR waveform preprocessing and peak detection. Raw ABR traces were filtered and baseline-corrected prior to peak identification. **(B)** Representative ABR waveforms recorded from PS19 mice and wildtype (WT) controls, and ApoE4/PS19 double transgenic mice and ApoE4 controls at 3, 7, and 10 months of age. Traces represent responses to tone stimuli presented at increasing sound intensities from 10 to 80 dB SPL, displayed as stacked waveforms. Each color represents a different stimulus intensity level. Vertical dashed lines visually identify peak I within the sample raw waveforms.

Figure 3. Auditory brainstem responses show modest age-related changes in PS19 and wild-type (WT) mice. **(A)** Hearing thresholds (dB SPL) in response to pure tone stimuli in 3-month-old (3M-WT n=9, 7M-WT n=15) and 7-month-old mice (3M-PS19 n= 8, 7M-PS19 n=13). WT mice at 7 months exhibit higher hearing thresholds at 8, 16, and 24 kHz compared with the 3-

month cohort. Similarly, PS19 mice at 7 months show elevated hearing thresholds at 24 kHz relative to the 3-month cohort. **(B)** Peak IV-to-I amplitude ratio in 3- and 7-month-old mice. IV/I amplitude ratios remain comparable with age for both PS19 and wild-type (WT) mice. **(C)** Peak I amplitudes (μV) in PS19 and WT control mice at 3- and 7-month-old at 4, 12, and 24 kHz. Peak I amplitudes are reduced in 7-month-old mice compared with 3-month-old cohorts in both PS19 and WT mice across tested frequencies. **(D)** Peak II amplitudes (μV) in PS19 and WT control mice 3- and 7-month-old at 4, 12, and 24 kHz. Peak II amplitudes in WT mice declined with age across tested frequencies. PS19 mice show comparable peak amplitudes between 3 and 7-month-old mice. **(E)** Peak III amplitudes (μV) in PS19 and WT control mice 3- and 7-month-old at 4, 12, and 24 kHz. PS19 mice showed comparable Peak III amplitudes between age groups at all tested frequencies. In WT mice, amplitudes declined with age at 4 and 12 kHz but remained comparable at 24 kHz. **(F)** Peak IV amplitudes (μV) in PS19 and WT mice 3- and 7-month-old, at 4, 12, and 24 kHz. Peak IV amplitudes were reduced in 7-month-old mice relative to 3-month-old cohorts in both PS19 and WT mice across all tested frequencies. Data represent mean ± SEM. Asterisks indicate significant differences between genotype-matched control (brown) and PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 4. Auditory brainstem response reveals hearing impairments in PS19 mice. **(A-B)** Hearing thresholds (dB SPL) in response to pure tone stimuli in 3- and 7-month-old mice. At 3 months old, PS19 mice show elevated hearing thresholds at 8 kHz. At 7 months, hearing thresholds were comparable to wild-type (WT) mice. **(C)** Peak I amplitudes (μV) in 3 and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in PS19 mice are reduced relative to controls at 3 months and become comparable to WT mice at 7 months. **(D)** Peak II amplitudes (μV) in 3 and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in PS19 mice are

reduced at 3 months at all frequencies and increase at 4 and 12 kHz at 7 months compared to WT mice. **(E)** Peak III amplitudes (μV) in 3 and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in PS19 mice are reduced at 3 months at the tested frequencies and remain reduced at 12 and 24 kHz at 7 months. **(F)** Peak IV amplitudes (μV) in 3 and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in PS19 mice are reduced at 3 months at 4 and 12 kHz and remain reduced at 7 months for 4 kHz only. Data represent mean ± SEM. Asterisks indicate significant differences between age-matched control (brown) and PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 5. ABR peak latency is delayed in PS19 mice. **(A)** Peak I latency (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak I latencies in PS19 mice are comparable to those of wild-type (WT) mice at both ages across all frequencies. **(B)** Peak II latency (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. PS19 mice show delayed Peak II latencies at 3 months that persist at 7 months at 4 and 12 kHz. At 24 kHz, peak latencies are comparable to WT mice at 3 months and become delayed at 7 months. **(C)** Peak III latency (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. PS19 mice show delayed Peak III latencies at 3 months across frequencies, with delays persisting at 7 months at 4 and 12 kHz compared to WT mice. **(D)** Peak IV latency (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak IV latencies are delayed in PS19 mice at 3 months across shown frequencies and continue at 7 months at 4 and 12 kHz, while remaining comparable to WT mice at 24 kHz. Data represent mean ± SEM. Asterisks indicate significant differences between age-matched control (brown) and PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 6. ABR interpeak latency is delayed in PS19 mice. **(A)** Interpeak latency from Peak I to II (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. PS19 mice show delayed Peak I–II interpeak latencies at 3 and 7 months at 4 and 12 kHz. At 24 kHz, interpeak latencies are comparable at 3 months and become delayed at 7 months. **(B)** Interpeak latency from Peak II to III (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak II–III interpeak latencies in PS19 mice are comparable at 3 months and delayed at 7 months at 4 and 12 kHz, while remaining comparable to WT mice at 24 kHz. **(C)** Interpeak latency from Peak III to IV (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak III–IV interpeak latencies in PS19 remain comparable to WT mice at all other frequencies and ages. **(D)** Interpeak latency from Peak I to IV (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak I–IV interpeak latencies in PS19 mice are delayed at 3 and 7 months at 4 and 12 kHz and remain comparable to WT at 24 kHz. Data represent mean ± SEM. Asterisks indicate significant differences between age-matched control (brown) and PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 7. Auditory brainstem responses show age-related changes in ApoE4/PS19 and ApoE4 control mice. **(A)** Hearing thresholds (dB SPL) in response to pure tone stimuli in 3-month-old (3M-ApoE4 n=11, 3M-ApoE4/PS19 n=9) and 7-month-old mice (7M-ApoE4 n=15, 7M-ApoE4/PS19 n=17). ApoE4 control mice at 7 months exhibit elevated hearing thresholds at 4, 8, 12, 16, and 24 kHz compared with the 3-month cohort. Similarly, ApoE4/PS19 mice at 7 months exhibit elevated hearing thresholds at 8, 16, 24, and 32 kHz compared with the 3-month cohort. **(B)** Peak IV-to-I amplitude ratio in 3- and 7-month-old mice. IV/I amplitude ratios remain comparable with age for ApoE4 controls at the shown frequencies. IV/I amplitude ratios remain comparable with age for ApoE4/PS19 mice at 4, 12 kHz, and at 24 kHz. **(C)** Peak I amplitudes (μV) in ApoE4 and ApoE4/PS19 mice at 3- and 7-month-old at 4, 12, and 24 kHz. Peak I

amplitudes are reduced in 7-month-old mice compared with 3-month-old cohorts in both ApoE4/PS19 and ApoE4 control groups across all tested frequencies. **(D)** Peak II amplitudes (μV) in ApoE4 and ApoE4/PS19 mice at 3- and 7-month-old at 4, 12, and 24 kHz. Peak II amplitudes remain comparable with increasing age in both ApoE4/PS19 and ApoE4 control groups at 4 and 12 kHz. Peak II amplitudes were reduced in 7-month-old mice relative to 3-month-old cohorts in both ApoE4 and ApoE4/PS19 mice at 24 kHz. **(E)** Peak III amplitudes (μV) in ApoE4 and ApoE4/PS19 mice at 3- and 7-month-old at 4, 12, and 24 kHz. Peak III amplitudes are reduced in 7-month-old mice compared with 3-month-old cohorts in both ApoE4/PS19 and ApoE4 control groups across all tested frequencies. **(F)** Peak IV amplitudes (μV) in ApoE4 and ApoE4/PS19 mice at 3- and 7-month-old at 4, 12, and 24 kHz. Peak IV amplitudes remain comparable with increasing age in both ApoE4/PS19 and ApoE4 control groups across 4, 12, and 24 kHz. Data represents SEM. Asterisks indicate significant differences between genotype-matched ApoE4 (black) and ApoE4/PS19 (red) mice. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$, and ns denoting non-significant comparisons.

Figure 8. Auditory brainstem responses show hearing impairment in ApoE4/PS19 relative to PS19 and wild-type (WT) mice. **(A-B)** Hearing thresholds (dB SPL) in response to pure tone stimuli in 3-month-old and 7-month-old ApoE4/PS19, PS19, and WT mice. At 3 months of age, ApoE4/Ps19 mice display elevated hearing thresholds at 12 and 16 kHz compared with WT mice. Relative to Ps19 mice, ApoE4/Ps19 mice show lower hearing thresholds at 8 kHz but higher hearing thresholds at 16 kHz. At 7 months, ApoE4/PS19 mice exhibit higher hearing thresholds at 12, 16, and 24 kHz relative to PS19 mice. **(C-D)** Peak I and II amplitudes (μV) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in ApoE4/PS19 are reduced compared to PS19 and WT mice at 3 and 7 months across all tested frequencies. **(E)** Peak III amplitudes (μV) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in

ApoE4/PS19 mice are increased relative to PS19 mice at 3 months across all tested frequencies and reduced at 7 months at 4 kHz. Peak amplitudes in ApoE4/PS19 mice are increased relative to WT mice at 3 months at 24 kHz and reduced at 7 months at 4 and 12 kHz. **(F)** Peak IV amplitudes (μV) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak amplitudes in ApoE4/PS19 are reduced compared to PS19 and WT mice at 3 and 7 months across all tested frequencies. Data represent mean ± SEM. Asterisks indicate significant differences between age-matched ApoE4/PS19 (red) groups and WT (brown) or PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 9. ABR peak latency is shortened in ApoE4/PS19 mice relative to PS19 mice and wild-type (WT) mice. **(A)** Peak I latencies (ms) in 3- and 7- month-old mice at 4, 12, and 24 kHz. At 3 months, Peak I latencies in ApoE4/PS19 are comparable to WT and PS19 mice at 4 and 12 kHz and shortened at 24 kHz. At 7 months, Peak I latencies are shortened across all tested frequencies in ApoE4/PS19 mice. **(B)** Peak II latencies (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak II latencies in wild-type controls and PS19 mice are delayed relative to ApoE4/PS19 mice at 3 and 7 months across all tested frequencies. **(C-D)** Peak III and IV latencies (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak latencies in ApoE4/PS19 are shortened compared to WT and PS19 mice at 3 and 7 months across all tested frequencies. Data represent mean ± SEM. Asterisks indicate significant differences between age-matched ApoE4/PS19 (red) groups and WT (brown) or PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 10. ABR interpeak latency is shortened in ApoE4/PS19 mice relative to PS19 mice and wild-type (WT) mice. **(A)** Peak I-II interpeak latencies (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak I-II interpeak latencies are shortened in ApoE4/PS19 mice compared to WT and PS19 mice at 3 and 7 months across all tested frequencies. **(B)** Peak II-III interpeak latencies (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak II-III interpeak latencies are shortened in ApoE4/PS19 mice compared to WT and PS19 mice at 3 and 7 months across all tested frequencies. **(C)** Peak III- IV interpeak latencies (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak III-IV interpeak latencies are shortened in ApoE4/PS19 mice compared to WT and PS19 mice at 3 and 7 months across all tested frequencies. **(D)** Peak I-IV interpeak latencies (ms) in 3- and 7-month-old mice at 4, 12, and 24 kHz. Peak I-IV interpeak latencies are shortened in ApoE4/PS19 mice compared to WT and PS19 mice at 3 and 7 months across all tested frequencies. Data represent mean ± SEM. Asterisks indicate significant differences between age-matched ApoE4/PS19 (red) groups and WT (brown) or PS19 (blue) groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

Figure 11. Comparative analysis of anxiety-related, and motor function in PS19 and ApoE4/PS19 mouse models of Alzheimer's disease. **(A)** Elevated plus maze (EPM) performance in PS19 mice at 3, 7, and 10 months of age. The time spent in closed arms and center zone show comparable anxiety-related behaviors in PS19 mice across age relative to wild-type (WT) mice at 3, and 7months old. At 10 months, PS19 spent more time in open arms compared to WT mice. **(B)** Rotarod performance in 3, 7, and 10-month-old PS19 mice. The latencies to fall (s) remain comparable in PS19 and WT mice across the tested ages. **(C)** Elevated Plus Maze (EPM) test results show anxiogenic behavior in ApoE4/PS19 mice. ApoE4/PS19 mice show reduced time spent in the closed arm and increased time spent in the

center zone compared to the ApoE4 controls at 3 and 10 months. **(D)** Rotarod performance shows changes in motor functions in 7-month-old ApoE4/PS19 mice. The latencies to fall (s) show comparable performance in ApoE4/PS19 and ApoE4 controls at 3 months old. At 7 and 10 months, ApoE4/PS19 mice exhibit greater latencies. Bar plots show similar motor performance for both groups at all measured ages. Data are presented as mean ± SEM, with asterisks indicating significant differences between control (brown) and mutant groups: PS19 (blue), ApoE4 (black), and ApoE4/PS19 (red). *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$ and ns denoting non-significant comparisons.

	Age-Effect on ABR				
	3M vs 7M - Statistical Test Results				
	Hearing Thresholds				
	Frequency (kHz)	WT Controls	PS19	ApoE4	ApoE4/PS19
	4	1.26×10^{-1}	4.73×10^{-1}	3.75×10^{-3}	2.42×10^{-1}
	8	1.19×10^{-2}	5.54×10^{-1}	9.65×10^{-3}	3.76×10^{-3}
	12	1.26×10^{-1}	2.36×10^{-1}	6.85×10^{-3}	5.99×10^{-2}
	16	1.36×10^{-2}	1.45×10^{-1}	1.23×10^{-3}	5.26×10^{-3}
	24	1.58×10^{-2}	4.87×10^{-2}	2.62×10^{-3}	5.87×10^{-4}
	32	1.45×10^{-1}	2.84×10^{-1}	9.09×10^{-2}	7.64×10^{-3}

Frequency (kHz)	Peak	Amplitude							
		WT Controls		PS19		ApoE4		ApoE4/PS19	
		LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val
4	I	4.75×10^{-9}	7.12×10^{-9}	2.31×10^{-4}	7.44×10^{-4}	1.09×10^{-5}	1.26×10^{-5}	2.44×10^{-20}	7.32×10^{-20}
	II	4.40×10^{-8}	2.64×10^{-7}	2.26×10^{-1}	3.39×10^{-1}	2.64×10^{-1}	3.17×10^{-1}	8.48×10^{-1}	8.48×10^{-1}
	III	9.00×10^{-7}	2.70×10^{-6}	3.43×10^{-1}	6.86×10^{-1}	8.26×10^{-14}	1.65×10^{-13}	8.19×10^{-18}	1.64×10^{-17}
	IV	1.43×10^{-12}	8.59×10^{-12}	5.17×10^{-15}	3.10×10^{-14}	2.52×10^{-1}	4.42×10^{-1}	4.35×10^{-1}	5.33×10^{-1}
12	I	1.31×10^{-9}	6.62×10^{-9}	2.48×10^{-4}	7.44×10^{-4}	5.01×10^{-13}	1.00×10^{-12}	2.40×10^{-20}	7.32×10^{-20}
	II	1.55×10^{-7}	4.64×10^{-7}	7.34×10^{-1}	7.34×10^{-1}	1.01×10^{-1}	2.02×10^{-1}	4.51×10^{-1}	6.27×10^{-1}
	III	1.10×10^{-4}	2.20×10^{-4}	5.21×10^{-2}	3.12×10^{-1}	1.25×10^{-11}	1.87×10^{-11}	6.99×10^{-20}	2.10×10^{-19}
	IV	9.50×10^{-11}	1.77×10^{-10}	1.22×10^{-8}	2.43×10^{-8}	7.82×10^{-3}	2.34×10^{-1}	5.33×10^{-1}	5.33×10^{-1}

24	I	4.65×10^{-5}	5.59×10^{-5}	4.28×10^{-3}	7.43×10^{-3}	1.11×10^{-5}	1.26×10^{-5}	1.84×10^{-7}	2.20×10^{-7}
	II	2.42×10^{-3}	2.90×10^{-3}	1.97×10^{-1}	3.39×10^{-1}	9.38×10^{-3}	5.63×10^{-2}	9.89×10^{-7}	5.93×10^{-6}
	III	2.53×10^{-1}	2.53×10^{-1}	8.52×10^{-1}	8.91×10^{-1}	1.73×10^{-25}	1.04×10^{-24}	2.54×10^{-14}	3.82×10^{-14}
	IV	2.59×10^{-5}	3.88×10^{-5}	5.84×10^{-8}	7.01×10^{-8}	5.17×10^{-1}	5.65×10^{-1}	2.12×10^{-1}	4.25×10^{-1}

933

934 **Table 1.** Statistical comparisons of auditory brainstem response (ABR) measures evaluating the
935 effect of age (3 vs. 7 months) in wild-type (WT), PS19, ApoE4, and ApoE4/PS19 mice. P-values
936 were obtained using linear mixed-effects (LME) models and corrected for multiple comparisons
937 using the false discovery rate (FDR).

938

	Genotype-Effect on ABR						
	3 months						
	PS19 vs WT Controls Statistical Analysis						
	Hearing Threshold						
	Frequency (kHz)	4	8	12	16	24	32
		1.13×10^{-1}	1.05×10^{-3}	2.15×10^{-1}	5.75×10^{-2}	1.57×10^{-1}	8.78×10^{-1}

		Amplitude		Latency		Interpeak Latency	
Frequency (kHz)	Peak	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val
4	I	7.01×10^{-3}	1.05×10^{-2}	1.34×10^{-1}	2.03×10^{-1}	1.41×10^{-9}	8.45×10^{-9}
	II	1.00×10^{-4}	3.01×10^{-4}	1.15×10^{-7}	6.88×10^{-7}	2.58×10^{-1}	5.16×10^{-1}
	III	4.90×10^{-5}	9.80×10^{-5}	9.45×10^{-5}	5.67×10^{-4}	1.62×10^{-2}	9.72×10^{-2}
	IV	1.26×10^{-3}	3.78×10^{-3}	3.00×10^{-25}	1.80×10^{-24}	4.61×10^{-36}	2.76×10^{-35}
12	I	3.70×10^{-3}	7.41×10^{-3}	4.49×10^{-1}	5.39×10^{-1}	6.02×10^{-3}	9.02×10^{-3}
	II	8.03×10^{-4}	1.39×10^{-3}	1.11×10^{-2}	1.67×10^{-2}	6.29×10^{-1}	6.29×10^{-1}
	III	1.69×10^{-4}	1.70×10^{-4}	9.22×10^{-3}	1.38×10^{-2}	6.46×10^{-2}	1.94×10^{-1}
	IV	2.81×10^{-4}	1.69×10^{-3}	9.27×10^{-6}	1.85×10^{-5}	2.24×10^{-9}	6.72×10^{-9}
24	I	3.96×10^{-2}	4.75×10^{-2}	1.35×10^{-1}	2.03×10^{-1}	4.86×10^{-1}	4.86×10^{-1}
	II	2.70×10^{-2}	3.24×10^{-2}	1.61×10^{-1}	1.61×10^{-1}	1.05×10^{-1}	5.16×10^{-1}
	III	1.70×10^{-4}	1.70×10^{-4}	1.41×10^{-2}	1.69×10^{-2}	5.52×10^{-1}	6.63×10^{-1}
	IV	7.73×10^{-1}	7.80×10^{-1}	4.43×10^{-2}	4.43×10^{-2}	8.19×10^{-2}	8.19×10^{-2}

	7 months
	PS19 vs WT Controls Statistical Analysis
	Hearing Threshold

	Frequency (kHz)	4	8	12	16	24	32
		9.63×10^{-1}	8.52×10^{-1}	9.26×10^{-1}	5.63×10^{-1}	4.84×10^{-1}	3.17×10^{-1}

		Amplitude		Latency		Interpeak Latency	
Frequency (kHz)	Peak	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val
4	I	1.18×10^{-1}	3.81×10^{-1}	8.69×10^{-2}	2.42×10^{-1}	1.41×10^{-3}	8.45×10^{-3}
	II	4.04×10^{-3}	1.16×10^{-2}	6.29×10^{-7}	3.77×10^{-6}	1.17×10^{-3}	2.73×10^{-3}
	III	4.06×10^{-1}	4.88×10^{-1}	1.88×10^{-6}	3.76×10^{-6}	8.10×10^{-1}	9.81×10^{-1}
	IV	1.73×10^{-5}	1.04×10^{-4}	4.51×10^{-6}	9.02×10^{-6}	9.80×10^{-6}	1.96×10^{-5}
12	I	3.29×10^{-1}	4.94×10^{-1}	4.24×10^{-1}	5.09×10^{-1}	2.07×10^{-2}	2.92×10^{-2}
	II	5.79×10^{-3}	1.16×10^{-2}	3.67×10^{-3}	5.51×10^{-3}	5.18×10^{-4}	2.73×10^{-3}
	III	9.85×10^{-4}	2.95×10^{-3}	2.15×10^{-7}	6.86×10^{-7}	8.43×10^{-1}	9.81×10^{-1}
	IV	4.89×10^{-2}	7.33×10^{-2}	4.54×10^{-5}	6.81×10^{-5}	4.40×10^{-6}	1.32×10^{-5}
24	I	5.57×10^{-1}	6.35×10^{-1}	7.76×10^{-1}	7.76×10^{-1}	2.44×10^{-2}	2.92×10^{-2}
	II	9.77×10^{-1}	9.77×10^{-1}	4.26×10^{-2}	4.26×10^{-2}	3.16×10^{-1}	3.16×10^{-1}
	III	6.16×10^{-3}	9.24×10^{-3}	2.57×10^{-1}	2.57×10^{-1}	1.74×10^{-1}	5.22×10^{-1}
	IV	6.31×10^{-2}	7.59×10^{-2}	7.87×10^{-1}	7.87×10^{-1}	8.77×10^{-1}	2.77×10^{-1}

Table 2. ABR statistical results of genotype-specific comparisons for PS19 vs WT mice at 3 and 7 months of age. All p-values were derived from linear mixed-effects (LME) models and adjusted for multiple comparisons using the false discovery rate (FDR).

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	Genotype-Effect on ABR						
	ApoE4/PS19 vs WT Statistical Analysis						
	Hearing Threshold						
	Frequency (kHz)	4	8	12	16	24	32
		9.80×10^{-2}	6.53×10^{-1}	3.60×10^{-2}	1.10×10^{-2}	7.72×10^{-2}	8.05×10^{-1}

		Amplitude		Latency		Interpeak Latency	
Frequency (kHz)	Peak	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val
4	I	4.37×10^{-33}	1.31×10^{-32}	8.12×10^{-1}	8.12×10^{-1}	1.01×10^{-113}	2.03×10^{-113}
	II	5.57×10^{-19}	1.11×10^{-18}	6.88×10^{-89}	4.13×10^{-88}	8.54×10^{-3}	1.03×10^{-2}
	III	3.26×10^{-1}	7.34×10^{-1}	2.54×10^{-52}	5.07×10^{-52}	2.50×10^{-23}	3.00×10^{-23}
	IV	2.28×10^{-86}	3.42×10^{-86}	1.45×10^{-254}	4.36×10^{-254}	3.01×10^{-302}	6.02×10^{-302}
12	I	6.45×10^{-30}	1.29×10^{-29}	7.62×10^{-2}	1.14×10^{-1}	8.10×10^{-116}	2.43×10^{-115}
	II	1.10×10^{-34}	3.30×10^{-34}	6.33×10^{-59}	1.27×10^{-58}	5.18×10^{-9}	1.04×10^{-8}
	III	8.18×10^{-1}	9.35×10^{-1}	8.44×10^{-74}	2.53×10^{-73}	4.81×10^{-37}	1.44×10^{-36}
	IV	1.41×10^{-102}	8.43×10^{-102}	1.23×10^{-225}	2.46×10^{-225}	1.00×10^{-308}	1.00×10^{-308}
24	I	1.87×10^{-16}	2.24×10^{-16}	2.06×10^{-15}	1.23×10^{-14}	1.76×10^{-29}	2.11×10^{-29}
	II	1.91×10^{-161}	1.15×10^{-160}	2.72×10^{-4}	3.26×10^{-4}	2.33×10^{-11}	1.40×10^{-10}
	III	1.69×10^{-3}	1.01×10^{-2}	4.96×10^{-26}	5.96×10^{-26}	2.18×10^{-26}	3.27×10^{-26}
	IV	2.23×10^{-45}	2.67×10^{-45}	1.36×10^{-76}	2.05×10^{-76}	2.68×10^{-130}	4.02×10^{-130}

	ApoE4/PS19 vs PS19 Statistical Analysis						
	Hearing Threshold						
	Frequency (kHz)	4	8	12	16	24	32
		3.93×10^{-1}	2.43×10^{-2}	1.68×10^{-1}	1.65×10^{-2}	5.24×10^{-1}	3.44×10^{-1}

		Amplitude		Latency		Interpeak Latency	
Frequency (kHz)	Peak	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val
4	I	5.27×10^{-47}	1.58×10^{-46}	1.04×10^{-1}	2.07×10^{-1}	1.77×10^{-153}	5.30×10^{-153}
	II	5.54×10^{-13}	8.31×10^{-13}	3.00×10^{-145}	1.80×10^{-144}	1.37×10^{-3}	1.37×10^{-3}
	III	1.49×10^{-4}	1.49×10^{-4}	7.81×10^{-119}	2.34×10^{-118}	3.80×10^{-31}	5.70×10^{-31}
	IV	1.08×10^{-103}	2.16×10^{-103}	1.00×10^{-308}	1.00×10^{-308}	1.00×10^{-308}	1.00×10^{-308}
12	I	8.75×10^{-44}	1.75×10^{-43}	3.46×10^{-1}	4.15×10^{-1}	3.57×10^{-110}	7.15×10^{-110}
	II	7.96×10^{-21}	2.39×10^{-20}	4.57×10^{-79}	9.14×10^{-79}	1.67×10^{-34}	1.00×10^{-33}
	III	1.05×10^{-6}	1.58×10^{-6}	1.23×10^{-144}	7.39×10^{-144}	4.12×10^{-46}	2.47×10^{-45}
	IV	2.54×10^{-107}	7.62×10^{-107}	1.00×10^{-308}	1.00×10^{-308}	1.00×10^{-308}	1.00×10^{-308}
24	I	1.79×10^{-20}	2.15×10^{-20}	7.88×10^{-20}	4.73×10^{-19}	2.70×10^{-33}	3.24×10^{-33}
	II	1.86×10^{-3}	2.23×10^{-3}	1.11×10^{-10}	1.11×10^{-10}	1.21×10^{-15}	3.64×10^{-15}
	III	6.48×10^{-24}	3.89×10^{-23}	3.52×10^{-61}	3.52×10^{-61}	3.66×10^{-41}	7.32×10^{-41}
	IV	3.01×10^{-66}	3.62×10^{-66}	5.52×10^{-109}	6.63×10^{-109}	1.00×10^{-308}	1.00×10^{-308}

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Table 3. ABR statistical results of genotype-specific comparisons for ApoE4/PS19 vs WT and ApoE4/PS19 vs PS19 mice at 3 months of age. All p-values were derived from linear mixed-effects (LME) models and adjusted for multiple comparisons using the false discovery rate (FDR).

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	Genotype-Effect on ABR							
	ApoE4/PS19 vs WT Statistical Analysis							
	Hearing Threshold							
	Frequency (kHz)	4	8	12	16	24	32	
		5.68×10^{-1}	6.46×10^{-1}	2.39×10^{-1}	2.29×10^{-1}	5.73×10^{-1}	3.69×10^{-1}	
		Amplitude		Latency		Interpeak Latency		
Frequency (kHz)	Peak	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	
4	I	1.15×10^{-50}	6.92×10^{-50}	7.95×10^{-20}	1.19×10^{-19}	6.38×10^{-52}	1.91×10^{-51}	
	II	1.29×10^{-9}	2.59×10^{-9}	6.45×10^{-8}	1.29×10^{-7}	2.06×10^{-7}	3.10×10^{-7}	
	III	1.17×10^{-4}	3.51×10^{-4}	1.22×10^{-15}	1.84×10^{-15}	2.24×10^{-38}	6.72×10^{-38}	
	IV	1.77×10^{-87}	1.06×10^{-86}	2.95×10^{-45}	8.86×10^{-45}	7.12×10^{-105}	2.13×10^{-104}	
12	I	5.13×10^{-39}	1.54×10^{-38}	4.53×10^{-19}	5.44×10^{-19}	1.30×10^{-65}	7.80×10^{-65}	
	II	5.96×10^{-10}	1.79×10^{-9}	6.67×10^{-15}	4.00×10^{-14}	2.55×10^{-15}	1.53×10^{-14}	
	III	3.71×10^{-5}	2.22×10^{-4}	7.25×10^{-38}	4.35×10^{-37}	1.43×10^{-40}	8.61×10^{-40}	
	IV	5.33×10^{-70}	1.60×10^{-69}	5.65×10^{-79}	3.39×10^{-78}	2.44×10^{-153}	1.46×10^{-152}	
24	I	5.49×10^{-16}	6.58×10^{-16}	6.60×10^{-9}	6.60×10^{-9}	1.42×10^{-15}	1.71×10^{-15}	
	II	8.72×10^{-4}	8.72×10^{-4}	6.13×10^{-1}	6.13×10^{-1}	2.65×10^{-11}	5.30×10^{-11}	
	III	4.89×10^{-2}	5.87×10^{-2}	2.12×10^{-4}	2.54×10^{-4}	9.00×10^{-16}	1.08×10^{-15}	
	IV	2.79×10^{-47}	3.34×10^{-47}	2.79×10^{-10}	3.35×10^{-10}	1.30×10^{-44}	1.56×10^{-44}	
	ApoE4/PS19 vs PS19 Statistical Analysis							
	Hearing Threshold							

	Frequency (kHz)	4	8	12	16	24	32
		7.56×10^{-2}	6.47×10^{-1}	3.62×10^{-2}	1.50×10^{-2}	4.18×10^{-2}	4.71×10^{-2}

		Amplitude		Latency		Interpeak Latency	
Frequency (kHz)	Peak	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val	LME p-val	FDR adjusted p-val
4	I	3.03×10^{-79}	1.82×10^{-78}	7.83×10^{-14}	1.17×10^{-13}	5.04×10^{-70}	1.01×10^{-69}
	II	1.46×10^{-16}	2.92×10^{-16}	2.99×10^{-17}	5.99×10^{-17}	5.20×10^{-14}	7.80×10^{-14}
	III	3.01×10^{-3}	9.04×10^{-3}	4.58×10^{-34}	9.17×10^{-34}	1.87×10^{-41}	1.12×10^{-40}
	IV	1.65×10^{-97}	4.94×10^{-97}	5.63×10^{-77}	1.69×10^{-76}	2.27×10^{-139}	6.80×10^{-139}
12	I	3.36×10^{-47}	6.72×10^{-47}	3.79×10^{-16}	7.58×10^{-16}	3.27×10^{-73}	9.82×10^{-73}
	II	1.64×10^{-14}	1.96×10^{-14}	2.52×10^{-21}	7.56×10^{-21}	4.27×10^{-25}	2.56×10^{-24}
	III	4.97×10^{-1}	4.97×10^{-1}	1.10×10^{-49}	6.58×10^{-49}	3.36×10^{-37}	1.01×10^{-36}
	IV	5.33×10^{-80}	1.07×10^{-79}	1.87×10^{-88}	1.12×10^{-87}	1.33×10^{-160}	7.99×10^{-160}
24	I	1.67×10^{-20}	2.01×10^{-20}	3.81×10^{-11}	4.57×10^{-11}	9.36×10^{-31}	1.12×10^{-30}
	II	2.61×10^{-4}	2.61×10^{-4}	2.76×10^{-2}	3.31×10^{-2}	2.16×10^{-8}	2.59×10^{-8}
	III	2.09×10^{-1}	3.14×10^{-1}	5.01×10^{-7}	6.01×10^{-7}	8.48×10^{-10}	1.02×10^{-9}
	IV	3.66×10^{-25}	4.39×10^{-25}	4.31×10^{-15}	4.31×10^{-15}	3.02×10^{-66}	3.63×10^{-66}

Table 4. ABR statistical results of genotype-specific comparisons for ApoE4/PS19 vs WT, and ApoE4/PS19 vs PS19 mice at 7 months of age. All p-values were derived from linear mixed-effects (LME) models and adjusted for multiple comparisons using the false discovery rate (FDR).

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	Behavior Statistical Results		
	EPM Statistical Results - PS19 Mouse Model		
	Open	Center	Closed
	WT Control vs. PS19	WT Control vs. PS19	WT Control vs. PS19
	3 Months		
Mann-Whitney Test	9.14×10^{-2}	3.17×10^{-1}	4.56×10^{-1}
	7 Months		
Mann-Whitney Test	3.69×10^{-1}	8.65×10^{-1}	6.35×10^{-1}
	10 Months		
Mann-Whitney Test	1.95×10^{-2}	8.59×10^{-1}	1.40×10^{-2}

	EPM Statistical Results - ApoE4/PS19 Mouse Model								
	Open			Center			Closed		
	ApoE4 vs. PS19/ ApoE4	ApoE4 vs. WT	PS19/ ApoE4 vs. WT	ApoE 4 vs. PS19/ ApoE 4	ApoE4 vs. WT	PS19/ ApoE4 vs. WT	ApoE 4 vs. PS19/ ApoE 4	ApoE4 vs. WT	PS19/ ApoE4 vs. WT
	3 Months								
Kruskal-Wallis Test	6.35×10^{-1}			2.30×10^{-3}			4.00×10^{-3}		
Dunn's Multiple Comparison	>0.9999	>0.9999	>0.9999	3.30×10^{-3}	>0.9999	1.73×10^{-2}	3.20×10^{-3}	>0.9999	7.86×10^{-2}
	7 Months								
Kruskal-Wallis Test	2.86×10^{-1}			3.75×10^{-1}			1.63×10^{-1}		

Dunn's Multiple Comparison	>0.9999	3.64 x 10^{-1}	9.17 x 10^{-1}	9.52 x 10^{-1}	5.19 x 10^{-1}	>0.9999	5.99 x 10^{-1}	1.83 x 10^{-1}	>0.9999
	10 Months								
Kruskal-Wallis Test	2.38 x 10^{-1}			7.00 x 10^{-4}			3.86 x 10^{-2}		
Dunn's Multiple Comparison	4.56 x 10^{-1}	>0.9999	3.79 x 10^{-1}	2.20 x 10^{-3}	4.00 x 10^{-3}	>0.9999	3.33 x 10^{-2}	4.16 x 10^{-1}	7.62 x 10^{-1}

	Rotarod Statistical Results				
	PS19 vs. WT		ApoE4 vs. PS19/ApoE4	ApoE4 vs. WT	PS19/ApoE4 vs. WT
	3 Months				
Mann-Whitney Test	8.78 x 10^{-1}	Kruskal-Wallis	2.90 x 10^{-1}		
		Dunn's Multiple Comparison	>0.9999	3.53 x 10^{-1}	9.87 x 10^{-1}
	7 Months				
Mann-Whitney Test	>0.9999	Kruskal-Wallis	<1.00 x 10^{-4}		
		Dunn's Multiple Comparison	3.20 x 10^{-3}	4.21 x 10^{-1}	<1.00 x 10^{-4}
	10 Months				
Mann-Whitney Test	5.99 x 10^{-1}	Kruskal-Wallis	3.00 x 10^{-4}		
		Dunn's Multiple Comparison	1.06 x 10^{-2}	>0.9999	4.00 x 10^{-4}

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Table 5. Table showing behavioral statistical results for PS19 vs WT, ApoE4/PS19 vs ApoE4, ApoE4 vs WT, and ApoE4/PS19 vs WT mice at 3, 7, and 10 months of age. Elevated plus maze uses the Mann-Whitney test for the PS19 mouse model and the Kruskal-Wallis test, followed by Dunn's multiple comparisons for the ApoE4/PS19 mouse models. Rotarod uses the Mann-Whitney test for the PS19 mouse model and Kruskal-Wallis, followed by Dunn's multiple comparisons for the ApoE4/PS19 models.