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Hearing loss is an early biomarker in APP/PS1 Alzheimer's disease mice

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a progressive loss of memory and cognitive decline. Over the last decade, it has been found that defects in sensory systems could be highly associated with AD. Hearing is an important neural sense. However, little is known about hearing functional changes in AD. In this study, APP/PS1 AD mice (Jackson Lab: Stock No. 004462) were used. Hearing function was assessed by auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and cochlear microphonics (CM) recordings. Wild-type (WT) littermates served as control. We found that APP/PS1 AD mice measured as ABR threshold had hearing loss. The hearing loss appeared at high frequency as early as 2 months old, prior to the reported occurrence of spatial learning deficit at 6–7 months of age in this AD mouse model. The hearing loss was progressive and extended from high frequency to low frequency. At 3–4 months old, the hearing loss appeared in the whole-frequency range. Moreover, the wave IV and V in the super-threshold ABR were eliminated, indicating substantial impairment in inferior colliculus, nuclei of lateral lemniscus, and medial geniculate body in the upper brainstem. DPOAE in APP/PS1 AD mice was also reduced. However, there was no reduction in CM in APP/PS1 mice. These data demonstrate that unlike age-related hearing loss APP/PS1 AD

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mice have early onset of hearing loss. These data also suggest that hearing function testing could provide a simple, sensitive, non-invasive screen-tool for early detecting AD and localizing lesion.

Keywords

Alzheimer's disease; hearing loss; auditory brainstem response; auditory brainstem; early AD biomarker; preclinical AD; age-related hearing loss

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disease, characterized by amyloid- β (A β) plaque deposition, tau protein aggregation, and neuronal loss. Over the last decade, increasing evidence from epidemiological studies suggests that defects in sensory systems, including the olfactory, visual, or auditory systems, are highly associated with AD [1,2]. AD pathology appears in sensory associated areas before appearing in regions involving memory, such as the entorhinal and hippocampal areas. These neuropathological changes in the sensory associated areas can be identified before cognitive symptoms become apparent. Thus, investigation of functional changes in the sensory systems could provide early, non-invasive AD biomarkers, which will be crucial to improving diagnosis, prevention, and treatment of AD.

Hearing is an important neural sense. However, hearing functional change in AD is less studied and is an understudied area in AD research. Early studies demonstrated that characterized AD pathological changes (A β plaques, tau protein aggregation, and neural degeneration) have been found in the human auditory system as well in the brain of those [3]. Positive A β plaques and tau proteins were identified in the brainstem from the cochlear nucleus (CN), superior olivary complex (SOC), inferior colliculus (IC), and nuclei of lateral lemniscus (NLL), to medial geniculate body (MGB) along the afferent auditory pathway in the old AD patients [3–5]. Widespread neuron and synapse loss in the central nucleus of IC and ventral region of MGB has been identified, but not in the adjacent, non-auditory structures in aged AD patients (52–86 years old) [4,5]. It has also been reported that AD patients may have difficulty with dichotic listening [6–8], understanding speech in the presence of background noise [9,10], impairment in hearing sensitivity [8,11], and impaired sound location [9, 12–13].

However, the relationship between hearing dysfunction and AD still remains unclear. There are conflicting results in the literature. For example, some studies found changes in hearing thresholds or peripheral hearing sensitivity in AD patients [13–15], while others reported no changes in AD patients [10, 16]. Moreover, as an age-related disease, the relationship between AD and age-related hearing loss (ARHL) also remains unclear. In this study, we used the APP/PS1 transgenic mouse, which is a leading AD mouse model and has been widely used in AD studies, and recorded hearing function at different ages to investigate hearing function changes in AD and AD development.

Materials and Methods:

AD mice

APP/PS1 AD mice were purchased from Jackson Laboratory, USA (Stock No: 004462, mixed C57BL/6;C3H genetic background). APP/PS1 mice are double transgenic mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) [17]. These transgenes were detected on tail genomic DNA by PCR amplification using the following primers: wild-type forward: 5'-GTG TGA TCC ATT CCA TCA GC- 3'; Common: 5'-GGA TCT CTG AGG GGT CCA GT- 3'; Mutant forward: 5'-ATG GTA GAG TAA GCG AGA ACA CG- 3'. Mutant and WT mice generated 142 and 265 bps bands, respectively. Wild-type (WT) littermates were used as controls. All experimental procedures were conducted in accordance with the policies of the University of Kentucky Animal Care & Use Committee.

Auditory brainstem response recording

Auditory brainstem response (ABR) was recorded in a double-wall sound isolated chamber by use of a Tucker-Davis ABR workstation (Tucker-Davis Tech. Alachua, FL). As we previously reported [18,19], mice were anesthetized by intraperitoneal injection with a mixture of Ketamine and Xylazine (a stock solution: 8.5 ml saline+1 ml Ketamine+0.55 ml Xylazine) given at a dose of 0.1 ml/10 g body weight. Body temperature was maintained at 37–38°C by placing anesthetized mice on an isothermal pad (Deltaphase, model 39dp, Braintree Scientific Inc., Massachusetts). ABR was evoked by both clicks and series of tone bursts (4 – 40 kHz, 10–80 dB SPL, a 5dB-step) with an ES-1 high-frequency speaker (Tucker-Davis Tech. Alachua, FL). If mice had severe hearing loss, the ABR test at the intensity range of 70 – 110 dB SPL was used. To assess progressive changes and the early appearance of hearing loss, the ABR was recorded monthly at postnatal day P60, 90, 150, (i.e., 2, 3, 5 months) and so on.

Cochlear microphonics recording

Cochlear microphonics (CM) was recorded with the same electrode setting as ABR recording in a double-wall sound isolated chamber using a Tucker-Davis ABR workstation (Tucker-Davis Tech. Alachua, FL). As described in our previous reports [18, 20], CM was evoked by the tone bursts (8–40 kHz). The response signal was filtered with a band-pass filter from 3 kHz to 50 kHz to filter out ABR signals, and averaged by 250 times.

Distortion product otoacoustic emission measurement

For distortion product otoacoustic emission (DPOAE) recording, two plastic tubes were inserted into the external ear canal and sealed with an earplug. Two pure tones (f_1 and f_2 , $f_2/f_1 = 1.22$) were simultaneously delivered into the ear. The test frequencies were presented by a geometric mean of f_1 and f_2 [$f_0 = (f_1 \times f_2)^{1/2}$]. The intensity of f_1 (I_1) was set at 5 dB SPL higher than that of f_2 (I_2). The responses were averaged by 150 times [21–23].

ABR threshold determination, data processing, and statistical analysis:

As we previously reported [18, 19], ABR thresholds were recognized that the sound-evoked responses were just undetectable. Moreover, some of the ABR thresholds were further verified by house-programmed deep-learning neural model with convolutional neural network (CNN). The amplitude and latency of peak in ABR were also measured by a house-made program with MATLAB (Mathworks Inc. USA). For CM measurement, the recorded CM signal was processed with FFT to obtain its frequency-spectrum.

Data were plotted by SigmaPlot and statistically analyzed by SPSS v25.0 (SPSS Inc. Chicago, IL). Data were expressed as mean $\pm$ s.e.m. Student t tests were performed after assessment of normality (SigmaPlot v13, Systat Software, or SPSS, SPSS Inc). The two-tailed Student's t test was used. The threshold for significance was $\alpha = 0.05$.

Results:

Hearing loss in APP/PS1 AD mice

Fig. 1 shows hearing loss in APP/PS1 mice. ABR thresholds in APP/PS1 mice were significantly increased (Fig. 1). The ABR thresholds in APP/PS1 AD mice ($n=12$) at P90 (3 months) were 47.1 ± 4.10 , 48.3 ± 3.98 , 42.1 ± 3.87 , 51.3 ± 4.36 , 63.3 ± 3.84 , and 73.1 ± 4.42 dB SPL for click, 8, 16, 24, 32, and 40 kHz tone stimulations, respectively, while the ABR thresholds in the control WT littermates ($n=8$) were 33.1 ± 1.13 , 37.5 ± 2.67 , 28.1 ± 1.99 , 35.6 ± 2.30 , 48.8 ± 3.24 , and 53.8 ± 2.95 dB SPL. In comparison with the control WT littermates, the ABR thresholds in APP/PS1 AD mice were significantly increased by 10–20 dB SPL ($P < 0.05$ at 8 kHz and < 0.01 at others, t test, two-tail). The hearing loss appeared more apparent at high-frequency range. The ABR threshold at 40 kHz in APP/PS1 AD mice was increased by ~20 dB SPL (Fig. 1b).

Hearing loss in APP/PS1 mice with aging

Hearing loss in APP/PS1 mice also appeared progressive and increased as age increased (Fig. 2). At P60 (2 months) old, ABR threshold in APP/PS1 mice at 40 kHz was significantly increased (Fig. 2f). Then, hearing loss extended from high-frequency ranges to low-frequency ranges. At P90 (3 months) old, the hearing loss appeared in the whole-frequency range. At P150 (5 months) old, as ABR thresholds in control WT littermates had apparent increase in the high-frequency range (32 and 40 kHz) due to ARHL, there was no significant difference in ABR thresholds between APP/PS1 mice and WT littermates at 32 and 40 kHz (Fig. 2e&f).

Changes in ABR waveforms in APP/PS1 mice

ABR waveforms in APP/PS1 mice also had apparent changes (Fig. 3). Different waves in ABR reflect sound stimulation-evoked neural responses in different auditory centers in the brainstem. Wave I, II, and III are associated with responses in the auditory nerve (AN), CN, and SOC, respectively. The wave IV and V were produced by the evoked neural responses in the IC, NLL, and MGB. In comparison with WT mice, wave IV and V in APP/PS1 mice at P90 (3 months) old almost disappeared (Fig. 3a). Quantitative measure shows that the amplitudes of wave II, IV, and V in APP/PS1 mice were significantly reduced (Fig. 3c–g). In

comparison with WT mice, the amplitudes of wave II, IV, and V in APP/PS1 mice for high-intensity stimulation (> 85 dB SPL) were reduced by ~40%, 65%, and 60%, respectively.

In addition, the averaged trace of wave III in WT mice ($n=6$) appeared to have two peaks (Fig. 3a). Their latencies were 2.83 ± 0.16 and 3.15 ± 0.15 ms, respectively. In APP/PS1 mice ($n=10$), the wave III only appeared one peak; its latency was 2.55 ± 0.19 ms, shorter than that of WT (Fig. 3a). Wave I and II in APP/PS1 mice also had apparent changes (Fig. 3a). The latency of wave I in WT and APP/PS1 mice was 0.90 ± 0.03 and 0.86 ± 0.05 ms ($P<0.05$, t test, two-tail), respectively. The wave II in APP/PS1 mice also appeared delayed (Fig. 3a); the latency of wave II in WT and APP/PS1 mice was 1.56 ± 0.08 and 1.63 ± 0.10 ms ($P<0.05$, t test, two-tail), respectively.

Reduction of DPOAE in APP/PS1 AD mice

DPOAE in APP/PS1 mice was also reduced (Fig. 4). In comparison with WT mice ($n=6$), the DPOAE in APP/PS1 mice ($n=8$) at P90 (3 months) old was reduced by 10–25 dB. The reduction was apparent at middle and high frequency ranges. At 16 and 20 kHz, the reduction of DPOAE in APP/PS1 mice was 26.9 ± 7.21 and 19.6 ± 3.65 dB SPL ($P<0.01$, t test, two-tail), respectively (Fig. 4c). However, there was no significant difference in DPOAE at 4 and 8 kHz between APP/PS1 mice and WT mice ($P=0.3$ – 0.5 , t test, two-tail) at the age of P90 (3 months).

Normal CM in APP/PS1 AD mice

CM is the auditory receptor current and reflects mechano-electronic transduction procedure in hair cells. Fig. 5 shows that CM in APP/PS1 mice ($n=12$) had no apparent difference from WT mice ($n=8$) at P150 (5 months) old. The I-O function of CM in the APP/PS1 mice also had no significant change, although the CM in APP/PS1 mice appeared slight reduced ($P=0.3$ – 0.8 , t test, two-tail, Fig. 5b).

Discussion:

In this study, we investigated hearing function in APP/PS1 AD mice. We found that APP/PS1 AD mice had hearing loss, which was detectable as early as 2 months in the high-frequency region (Figs. 1–3). Then, hearing loss extended toward mild and low frequency regions and appeared significant across all frequencies at 3 months and afterward (Fig. 2). In particular, ABR wave IV and V, which originate from the responses of the upper auditory centers in the brainstem and midbrain, were markedly diminished (Fig. 3). DPOAE in APP/PS1 mice was also reduced (Fig. 4). However, auditory receptor current CM was normal in APP/PS1 mice (Fig. 5). These data indicate that APP/PS1 AD mice have early onset of hearing loss, and suggest that the AD pathology could occur early in the auditory neural pathway.

APP/PS1 AD mice have been widely used in AD study. It has been reported that the APP/PS1 mice usually develop A β -deposits in brain and appear typical AD phenotypes, such as spatial learning deficit, by 6–7 months of age [17, 24, 25]. In this study, we found that hearing loss in the APP/PS1 AD mice measured as ABR threshold could be found as early as 2–3 months (Figs. 2–3). This is much earlier than the occurrence of other AD

phenotypes at 6–7 months old [17, 24, 25]. Moreover, we found that ABR wave IV and V in the APP/PS1 mice at 3 months old had significant reduction and were almost abolished (Fig. 3), indicating that the upper auditory centers of the IC, NLL, and MGB in the brainstem may have substantial impairment. Thus, these data suggest that hearing function testing might be able to provide an early, non-invasive biomarker for AD detection and lesion location and assessment.

Our finding that APP/PS1 mice have hearing loss is consistent with previous reports. It has been reported that 3xTg AD mice have increases in ABR threshold; spiral ganglion neurons, but not hair cells, are degenerated from 9 months old and thereafter [26]. Also, the 5xFAD mouse model has been found to have significant increases in ABR thresholds and apparent hair cell loss at 15–16 months old [27]. A recent study further demonstrated that the 5xFAD mice have early onset of gap detection deficits, which could be detectable at about 2 months old and became progressive worse [28]. In addition, the auditory startle response (ASR) was also recorded for behavioral assessment in some AD mouse models. It has been reported that some AD mice (e.g., Tg2576 and APP^{swe}/PS1M164L AD mice) had normal ASR and that some of them (e.g., 3xTg AD and CRND8 mice) showed exaggerated startle [29–31]. However, to our knowledge, there is no information available about hearing function of APP/PS1 AD mice.

In this study, we found that besides diminishment of wave IV and V, the latency of wave II in APP/PS1 AD mice was delayed and inter-wave I-II was extended at 3 months old (Fig. 3). Previous studies in human subjects also showed that inter-wave I-V of ABR in AD could be delayed [32, 33]. However, other studies failed to identify any significant changes in ABR in subjects with AD [10]. This diversity could arise from several reasons. First, AD human subjects in those studies might be at different stages of AD. Or, the AD stages were not well-defined (i.e., employed different diagnosing criteria). Second, the subjects might have different ages. It has been well-known that aging can induce ARHL. ARHL is estimated to affect up to 40% of those over the age of 65 and 75% in age >80 years [34,35]. In a previous report [10], the averaged ages of subjects in both AD and control groups were >75 years old. Apparently, ARHL could occur in those subjects even in control group without AD. Thus, AD-associated hearing loss and ARHL could be mixed together and no difference could be found in those old persons, or at the late-stage of AD. However, in this study, we found that hearing loss occurred early at 2 months old in APP/PS1 AD mice (Figs. 1–3), i.e., at the early-stage of AD. In this young age, ARHL could be apparent.

As age increased, the effect of aging in hearing function could be seen. In Fig. 2e&f, the ABR thresholds at high frequencies in WT littermates at 5 months old started to increase, since they have C57BL/6 background. C57BL/6 is a well-known ARHL mouse model and the ABR thresholds begin to be elevated at 3–6 months of age, starting at high frequencies [36, 37]. However, we used WT littermates as control in this study. Both WT and APP/PS1 mice have the same genetic background. Compared with the control WT littermates, APP/PS1 mice demonstrated hearing loss as early as 2 months old (Fig. 2). Thus, ARHL cannot play a major role in this early hearing loss, and auditory function tests could provide important information for assessing AD development and progression, in particular, in the early stage of AD (preclinical AD).

Currently, the prevalence of AD is increasing rapidly in the United States and world. It is estimated that there will be 13.8 million AD patients in the United States by the year 2050, almost triple the 4.7 million patients in 2010 [38]. AD is a progressive neurodegenerative disease. Early diagnosis and interventions delaying the onset of dementia by even one year would decrease the worldwide prevalence of dementia by 10%. Our study demonstrates that auditory function tests can provide an early, non-invasive biomarker for AD diagnosis. Moreover, compared with other sensory systems, the auditory system has the advantage that decline in function over time in different nuclei (centers) can easily be repeatedly, quantitatively measured and located by recordings of auditory evoked potentials. ABR is a routine auditory function test in the clinic and can assess the function and integrity of the auditory pathway from the spiral ganglion neurons to the auditory centers. Our study suggests that ABR recording could serve as a non-invasive, repeatable method to assess AD development and progression and lesion localization, in particular, in the early (preclinical) stage of AD.

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Abbreviations:

ABR	Auditory brainstem response
AD	Alzheimer's disease
ARHL	Age-related hearing loss
ASR	Auditory startle response
CM	Cochlear microphonics
DPOAE	distortion product otoacoustic emission
WT	Wild-type

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Highlights:

- APP/PS1 AD mice have progressive, early onset of hearing loss
- Hearing loss is detectable at 2-month old prior to other AD phenotypes
- Wave IV and V in ABR in APP/PS1 AD mice are minimized
- DPOAE is reduced but CM is normal in APP/PS1 AD mice

Fig. 1. Hearing loss in APP/PS1 AD mice. **a:** ABR waveforms were evoked by click stimulations in WT and APP/PS1 mice. **b:** Hearing loss in APP/PS1 mice. ABR thresholds were measured by click and tone bursts. Mice were P90 (3 months) old. WT littermates were used as control. *: $P<0.05$, **: $P<0.01$ (t test, two-tail).

Fig. 2.

Progressive hearing loss in APP/PS1 AD mice with age. WT littermates were used as control. ABR thresholds were measured by click and tone bursts. Inset at middle upside: A schematic drawing of the time sequence of occurrence of hearing loss and typical AD phenotypes in APP/PS1 AD mice. *: $P < 0.05$, **: $P < 0.01$ (t test, two-tail).

Fig. 3.

Reduction of amplitudes of wave IV-V of ABR in APP/PS1 mice. Mice were P90 (3 months) old. **a:** Average traces of ABR from APP/PS1 mice (n=10) and WT littermates (n=6). The traces were evoked by a click stimulus at 90 dB SPL and averaged. Bars represent SEM. Wave I-III are produced by the response of the auditory nerve (AN), the cochlear nucleus (CN), and superior olivary complex (SOC), respectively. Wave IV-V are produced by the activity of the inferior colliculus (IC), nuclei of lateral lemniscus (NLL), and medial geniculate body (MGB). **b-g:** The measured amplitudes of wave I-VI in APP/PS1 and WT mice. There are significant reductions in amplitudes of wave II, IV, V, and VI in APP/PS1 mice. *: P<0.05, **: P<0.01 (t test, two-tail).

Fig. 4.

Reduction of DPOAE in APP/PS1 AD mice. **a:** Spectrum of acoustic emission recorded from APP/PS1 AD and WT mice. Mice were around P90 (3 months) old. WT littermates served as control. $f_0=16$ kHz, $I_1/I_2=60/55$ dB SPL. **b:** I-O-functions of DPOAE in APP/PS1 AD mice and WT mice. There is significant difference in the I-O functions between APP/PS1 AD mice and WT mice. **c:** Decrease of DPOAE in APP/PS1 at different frequency. DPOAEs in APP/PS1 AD mice were referred to those in WT mice. *: $P<0.05$, **: $P<0.01$ (t test, two-tail).

Fig. 5. Normal CM in APP/PS1 AD mice. **a:** CM waveforms in APP/PS1 AD mice and WT mice evoked by 8 kHz tone bursts. **b:** I-O function of CM recorded from APP/PS1 AD mice and WT mice at P150 (5 months) old. There is no significant difference in the CM IO function between APP/PS1 AD and WT mice.