Early functional and cognitive declines measured by auditory evoked cortical potentials in Alzheimer's disease mice

Ling Mei*, Li-Man Liu*, Kaitian Chen, Hong-Bo Zhao

Department of Otolaryngology, University of Kentucky Medical Center, 800 Rose Street, Lexington, Kentucky, USA, 40536

* Equal contributors

Running title: Declining of AECP in AD mice

Corresponding Author:

Hong-Bo Zhao, Ph.D./M.D.
Professor
Dept. of Otolaryngology
University of Kentucky Medical Center
800 Rose Street
Lexington, KY 40536 – 0293
USA
Tel: 859-257-5097 x 82138
Fax: 859-257-5096
E-mail: hzhao2@uky.edu

Abstract

Alzheimer's disease (AD) is characterized with a progressive loss of memory and cognitive decline. However, assessment of AD-associated functional and cognitive changes is still a big challenge. Auditory evoked cortical potential (AECP) is an event-related potential reflecting not only neural activation in the auditory cortex but also cognitive activity in the brain. In this study, we recorded AECP changes in normal aging CBA/CaJ mice and APP/PS1 AD mice. AECP in mice usually possessed 3 waveforms, i.e., P1, P2, and P3 positive peaks and three corresponding negative peaks N1, N2, and N3. In normal aging mice, the early sensory peaks P1, N1, and P2 were reduced as age increased, whereas the later cognitive peaks P3 and N3 had no significant reduction. However, the latencies of peaks N2 and P3 were significantly reduced at 1 year old. In AD mice, peak P1 was significantly reduced in comparison with wild-type (WT) mice at young ages, proceeding occurrence of AD phenotypes. The later cognitive peak P3 was diminished invisible after 3 months old, different from normal aging effect. The latencies of AECP peaks in AD mice generally had no significant delay. Finally, consistent with AECP changes, expression of amyloid precursor protein (APP) was visible at the auditory cortex in AD mice at 2 months old. These data reveal that AECP could serve as an early, noninvasive, and objective biomarker to detect AD and AD-related dementia.

Keywords: AECP; event-related potential; dementia; early AD biomarker; preclinical AD; age-related hearing loss

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disease, characterized by a progressive loss of memory and dementia. Over the last two decades, the prevalence of AD and AD-related dementia (ADRD) has been rapidly increased. It is prospected that there will be 150 million AD patients by the year 2050, almost triple the population in 2018 (Hebert et al., 2013; Patterson et al., 2018). This will produce a big, heavy economic and social burden. Early detection is critical for prevention and interventions of this common neurodegenerative disease. It is estimated that early diagnosis and interventions delaying the onset of dementia by even one year could decrease the worldwide prevalence of dementia by 10% (Patterson et al., 2018). However, since lack of the early, reliable AD biomarkers, the early detection of AD currently is still a big challenge. In particular, it lacks objective biomarkers to detect cognitive decline, which is the most common and predominant symptom of AD, at the early stage.

Recently, increasing evidence suggests that defects in sensory systems, including the olfactory, visual, or auditory systems, are highly associated with AD progressing (Murphy, 2019; Rochoy et al., 2019). AD pathology could appear 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 occur before cognitive symptoms become apparent. Hearing is an important neural sense. AD can cause hearing loss; hearing loss also can in turn accelerate and exacerbate AD and dementia generation (Morrison et al., 2018; Griffiths et al., 2020). Our previous study demonstrated that auditory brainstem response (ABR), which reflects the function and

integrity of the auditory pathway in the brainstem, had apparent reduction in APP/PS1 AD mice at the age of 3 months old (Liu et al., 2020), prior to occurrence of spatial learning deficit in this AD mouse model by age of 6-7 months old (Reiserer et al., 2007; Ordonez-Gutierrez et al., 2015, 2016). Recent studies also demonstrated that 5xFAD mice have early onset of deficits in auditory gap detection (Kaylegian et al., 2019; Weible et al., 2020). These studies suggest that AD could cause hearing function decline at the early stage, and that hearing function tests could provide important information for detecting AD at the early stage.

Auditory evoked cortex potential (AECP) is an auditory evoked potential recorded on the scalp surface and reflects the neural activity in the auditory cortex and related brain areas. In particular, its later waveforms reflect cognitive processing (Sur and Sinha, 2009). In this study, we recorded AECP in APP/PS1 AD mice to assess AD-induced functional changes in the auditory cortex and brain. We found that AECP in AD mice could have significant reduction at young ages. In particular, later cognitive peak P3 was diminished in AD mice, different from normal aging effect. Our study suggests that AECP recording could serve as an early noninvasive biomarker to assess AD and ADRD.

Materials and Methods:

APP/PS1 AD mice and CBA/CaJ normal aging mice

APP/PS1 transgenic mice were used in this study and purchased from Jackson Laboratory, USA (Stock No: 004462, mixed C57BL/6;C3H genetic background). After received, we crossed them with CBA/CaJ mouse strain (Stock No: 00654, Jackson Laboratory, USA). APP/PS1 transgenic mice possess both chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and mutant human presenilin 1 (PS1-dE9) (Reiserer et al., 2007). Based on Jackson Laboratory's technical notice, APP/PS1 transgenes were detected by PCR amplification of tail genomic DNA using the following primers: wild-type (WT) 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. WT littermates served as controls. CBA/CAJ mice were also used for normal aging study. All experiments and procedures were approved and conducted in accordance with the policies of the University of Kentucky Animal Care & Use Committee (approved protocol #: UK00902M2005).

Auditory evoked cortical potential recording

Auditory evoked cortical potential (AECP) was recorded in a double-wall sound isolated chamber by use of a Tucker-Davis R3 system with an ES-1 high-frequency speaker (Tucker-Davis Tech. Alachua, FL). Mice were anesthetized by intraperitoneal injection with a mixture of ketamine and xylazine (8.5 ml saline+1 ml Ketamine+0.55 ml Xylazine, 0.1 ml/10 g). Body temperature was maintained at 37–38°C by placing

anesthetized mice on an isothermal pad. As the same as ABR recording, two subdermal needle electrodes were punched into the head skin at the vertex and ventrolaterally to the right or left ear. The ground needle electrode was inserted to the right leg. Cortical potentials were evoked by clicks (85 dB SPL) in alternative polarity with stimulating rate of 1/s. The recording time window was 800 ms, starting from the beginning of the stimulation. The signal was amplified by 50,000 and averaged by 200 time. The recording filter was set at 0 to 3 kHz. The signals were further post-filtered with a 10-order digital Gaussian filter with 12.5 Hz of central frequency and 2 Oct bandwidth for analysis.

Brain slide preparation

Mice were anesthetized with Ketamine as described above and was intracardially perfused with 4% paraformaldehyde for 15-20 min. After decapitation, the mouse skull was opened. The brain was carefully removed and incubated in 4% paraformaldehyde overnight for post-fixation. Following infiltration with 30% glucose for three day with changing fresh solution at each day, the brain was embedded in OCT (Cat # 4583, Sakura Finetek USA Inc. CA) at room temperature for one day. Then, the embedded brain was frozen at -80 °C overnight and cut 20 µm thick at -22~24 °C by a cryostat (Thermo Electron Corp. Waltham, MA). The tissue sections were directly mounted onto glass slides for staining and storage.

Immunofluorescent staining

Immunofluorescent staining was performed as described in our previous reports (Zhao and Yu, 2006; Wang et al., 2009). After 1 hr of incubation with the blocking solution (10% goat serum and 1% BSA in PBS) with 0.5% Triton X-100 at room temperature, the brain slides were incubated with primary antibody rabbit monoclonal anti-amyloid precursor protein (APP) [clone Y188] (#AB32136, Abcam, USA; 1:500) in blocking solution at 4 °C overnight. After washout with PBS three times, the brain slides were incubated with corresponding secondary antibody conjugated with Alexa Fluor-568 (Thermo Fisher Sci, USA) for 2 hr at room temperature. At the last 15 min, 4', 6-diamidino-2-phenylindole (DAPI, 0.1 mg/ml, D1306; Molecular Probes) was added into the incubation solution to visualize cell nuclei. After completely washing out the second antibodies with PBS, the sections were mounted with a fluorescence mounting medium H-1000, Vector Lab, CA) and observed under a Nikon A1R confocal microscope system. The AD and WT mouse sections were simultaneously stained and observed with the same parameters.

Data processing, statistical analysis, and reproducibility:

The amplitude and latency of peaks in AECP were measured by a house-made program with MATLAB (Mathworks Inc. USA). Data were plotted by SigmaPlot and statistically analyzed by SPSS v25.0 (SPSS Inc. USA). Data were expressed as mean ± s.e.m. Student t tests were performed between APP and WT groups at the defined age. When three or more groups were compared, one-way ANOVA with Bonferroni post hoc test was used. The threshold for significance was $\alpha = 0.05$. The recordings were repeated with different mice coming from at least three different breeding pairs.

Results:

Auditory evoked cortex potential (AECP) in mice

While recording of AECP is common in humans, little is recorded in mice. We first characterized AECP in mice. In mice, AECP usually appears 3 positive peaks, i.e., P1, P2, and P3, with corresponded three negative peaks N1, N2, and N3 (Fig. 1). Peak P1 and P2 could be clearly visible in recordings. However, P3 in mice was not visible in AECP until the age of 3 months old (Fig. 1A&B). At 6 months old, AECP was well-developed and P1, P2, and P3 peaks were clearly visible (Fig. 1C). The latencies of peaks P1, P2, and P3 were 13.5±1.89, 67.5±2.74, and 128.1±6.76 ms, respectively (Fig. 2A).

Fig. 1

Normal aging AECP changes in CBA/CaJ mice

We further investigated the effect of normal aging on AECP. CBA/CAJ mice, which have no apparent age-related hearing loss (Zheng et al., 1999), were used. Fig. 1 shows that AECPs were reduced with age increased. In comparison with those at the age of 3 months old, the amplitudes of early waveforms (i.e., P1, N1, and P2) had significant reduction and were 4.66±0.70, -4.73±0.71, and 3.15±0.75 μV, respectively, at 1 year old and 3.39±0.77, -3.98±0.78, and 3.41±0.86 μV, respectively, at 1.5 year (18 months) old (Fig. 2C&D). However, the amplitudes of later peaks N2, P3, and N3 had no significant reduction with aging (Fig. 2C&D). There were no significant changes in P1 and N1 latencies. However, the latencies of peaks P2, N2, and P3 were significantly reduced at 1 year old (Fig. 2B).

Fig. 2

AECP in APP/PS1 AD mice

Fig. 3 shows AECP traces recorded from APP/PS1 AD mice and WT littermates at different ages. In general, P1 and P2 in AECP in AD mice appeared smaller than those in WT littermate mice at young ages. At 1-3 months old, there was a notch at the descending side of peak P2 in AD mice (indicated by blue arrows in Fig. 3A-C). P3 is clearly visible in WT mice after 4 month old, but not visible in AD mice (Fig. 3D-I). Later negative peaks N2 and N3 were also not visible in the AECP in AD mice (Fig. 3D-I).

Fig. 3

Changes of AECP in APP/PS1 AD mice with aging

To assess dynamic changes with aging, we measured AECP recordings at different ages. Quantitative measurement shows that the early peak P1 in AD mice at 3-7 months old was significantly reduced in comparison with WT mice (Fig. 4A). Later cognitive peak P3 in AD mice was also significantly smaller than WT mice after 3 months old (Fig. 4E). However, N2 and N3 peaks in AD mice had no significant reduction in comparison with WT mice except 10-12 months old, at which N3 peak in AD mice was significantly reduced (Fig. 4F).

Fig. 4

The latencies of peak N1 in AD mice appeared shorter than those in WT mice at young ages (<7 months old) (Fig. 5B). The latencies of N3 in AD mice were also shorter than those in WT mice at 10-12 months old (Fig. 5F). However, the latency of N2 in AD mice appeared longer than that in WT mice at 12 months old (Fig. 5D). There was no significant difference in latencies of positive peaks between AD and WT mice except 3 months old (Fig. 5A), at which the P1 latency in AD mice was significantly shorter than that in WT mice.

Fig. 5

Different aging effects on AECP in AD mice and normal aging CBA/CaJ mice

We further examined difference between AD and normal aging effects on AECP in AD mice, WT littermate mice, and CBA mice (Fig. 6). Changes of AECP with normal aging in WT littermate mice and CBA mice were similar. However, the early waveform P1 in AD mice had significant reduction at 3 and 6 months old (Fig. 6A-C). Also, the later waveforms of N2, P3, and N3 in AD mice were significantly reduced at 3-12 months old (Fig. 6D-F).

Fig. 6

Fig. 7 shows the normal aging effect on AECP in CBA, WT littermates, and AD mice. In comparison with those peaks at the age of 3 months old in each group, the sensory P1 and N1 peaks in CBA and WT littermate mice had significant reduction with

aging (Fig. 7A&B). However, P1 and N1 peaks in AD mice had no significant reduction. The later cognitive waveforms N2, P3, and N3 were increased with aging in CBA and WT littermate mice but decreased in AD mice (Fig. 7D-F). AD and normal aging show different effects on AECP.

Fig. 7

Expression of amyloid precursor protein at the auditory cortex in APP/PS1 AD mice

Amyloid precursor protein (APP) is an AD protein. Immunofluorescent staining shows that the positive labeling of APP was visible at the auditory cortex in APP/PS1 AD mice at 2 months old (Fig. 8B&E), consistent with early changes in AECP recordings. As age increased, the deposition of APP in the auditory cortex was increased. At the age of 1 year old, the typical APP plaque deposition pattern was visible at the auditory cortex (Fig. 8A&D).

Fig. 8

Discussion:

In this study, we recorded AECP in mice and characterized changes of AECP in APP/PS1 AD mice and normal aging CBA/CaJ mice. We found that different from humans, AECP in mice usually had three waveforms (Fig. 1). P3 peak was not visible until 3 months old (Fig. 1B). As age increased, the early peaks (P1, N1, and P2) in AECP were reduced, while later peaks (i.e. N2, P3 and N3) had significant increase or no changes (Figs. 1, 2, and 7). In APP/PS1 AD mice, P1 peak at young ages (<8 months old) appeared smaller than those in WT mice (Figs. 3, 4, and 6). Peak P2 in AD mice at young age (1-3 months old) also had a notch in its descending side (Fig. 3A-C). In particular, the later cognitive peak P3 in AD mice was diminished (Figs. 3, 4, and 6). However, there was no significant peak delay in AD mice, except N2 at 12 months old (Fig. 5). Consistent with early changes in the AECP, expression of APP AD proteins was visible in the auditory cortex as early as 2 months old (Fig. 8). These data suggest that AECP recording could serve as an early, noninvasive biomarker to detect and assess AD generation and development.

AECP is an event-related potential reflecting the summation of post-synaptic potentials that are time-locked to certain events of interest. In humans, AECP consists of several waveforms and are named as P1 (P50), N1 (N100), P2 (P200), N2 (N250), P3 (P300), and P4 (P600) based on their latencies (Swords et al., 2018). Early waveforms (i.e., P1 & P2) are “sensory”, largely depending on the stimulus and activities in the auditory cortex; later waveforms (i.e., P3 & P4) are “cognitive” reflecting cognitive processing (Sur and Sinha, 2009). Thus, unlike other stimulus-evoked responses, the

AECP recording can reflect the neural activities in the auditory cortex and also cognitive activities in the brain.

Although AECP is a common and traditional recording in humans, little is recorded in mice. In this study, we found that AECP could be clearly evoked in mice by click stimulation. However, in comparison with the AECP recorded from humans, AECP in mice appeared only 3 waveforms (Fig. 1). Also, the latencies of P1, P2, and P3 in mice were much shorter comparing with humans and were in the range of 10-15, 65-70, and 110-150 ms, respectively (Fig. 2A&B). This may be due to the mouse brain size is small and the neural interactions are less than humans. In addition, P3 in mice was not well-developed until 3 months old (Fig. 1). As mentioned above, later responses of AECP reflect cognitive processing (Sur and Sinha, 2009). Specifically, P3 is dependent on internal thought process and is considered to be mainly generated by the hippocampus where short-term memory functions are stored. Thus, these data suggest that mouse cognition needs to take longer time for maturation.

In APP/PS1 AD mice, early sensory P1 peak at young ages (<8 months old) appeared small in comparison with those in WT mice (Figs. 3, 4, and 6). Peak P2 in AD mice at 1-3 months old had a notch at its descending side (Fig. 3A-C). These data demonstrated that AD could result into functional changes in the auditory cortex at the early AD stage. In previous study, we reported that hearing loss measured as ABR threshold in APP/PS1 AD mice could occur as early as 2-3 months old (Liu et al., 2020). The significant reduction was found in ABR peaks IV and V, which are generated by neural activities of

inferior colliculus, nuclei of lateral lemniscus, and medial geniculate body in the upper brainstem. It was also reported that the 5xFAD mice have early onset of deficits in auditory gap detection at 2 months old (Kaylegian et al., 2019; Weible et al., 2020). These data demonstrated that dysfunctions of hearing could occur much earlier than the typical AD phenotypes occurring. For example, spatial learning deficit, which is a typical AD symptom, occurs in APP/PS1 AD mice by age of 6-7 months old (Reiserer et al., 2007; Ordonez-Gutierrez et al., 2015, 2016). It has been reported that auditory sensory and cognitive cortical potentials in AD persons could be abnormal approximately 10 years before AD becomes apparent (Golob et al., 2009). Thus, AECP recording as well as ABR recording and other hearing function tests could provide important information for early AD detection.

Consistent with AECP recording, we found that expression of APP could occur in the auditory cortex as early as 2 months old (Fig. 7B&E). Previous studies in AD patients demonstrated that AD pathology including Aβ plaques, tau protein aggregation, and neural degeneration existed in the human brainstem and auditory cortex in old AD patients (Ohm and Braak, 1989; Sinha et al., 1993; Baloyannis et al., 2007, 2009). Our result is consistent with these previous reports and provide further morphological evidence that AD could occur in the auditory system at the early stage.

In this study, we found that peak P3 in AECP was significantly reduced in AD mice (Figs. 3, 4 and 6). As mentioned above, the peak P3 in AECP reflects cognitive neural processing. The previous studies found that AD patients could have difficulty with

dichotic listening (Grimes et al., 1985; Mohr et al., 1990; Strouse et al., 1995), understanding speech in the presence of background noise (Kurylo et al., 1993; Gates et al., 1995), impairment in hearing sensitivity (Strouse et al., 1995; Iliadou and Kaprinis, 2003), and impaired sound location (Strouse et al., 1995; Gates et al., 2002, 2011). However, there were conflicted results in previous studies, since most of those tests are based on subjective selection and judgment, which largely depend on personal cognitive conditions. Moreover, AD or aged persons have cognitive decline or dementia (Gate et al., 1995). This could dramatically affect those subjective-dependent functional tests and performance. Our study demonstrates that AECP recording can provide an objective test to assess cognitive decline and AD progression.

Currently, more and more AD mouse models are available. They provide useful animal models to study AD pathological mechanisms, develop therapeutic interventions, and assess the treatment efficiency. However, as mentioned above, it currently lacks reliable objective biomarkers to assess AD phenotypes, in particularly, cognitive decline in animals. In this study, we found that cognitive peak P3 of AECP in AD mice was diminished, different from normal aging effect (Figs. 3, 4, 6, and 7). These findings demonstrate that AECP recording could provide an objective, recordable, and repeatable biomarker to assess AD and cognitive decline in mice. This could greatly facilitate AD studies.

In this study, we also investigated the effect of normal aging on AECP. In normal aging CBA mice, early waveforms (P1, N1, and P2) in AECP had significant reduction as

age increased, whereas later waveform peaks N2, P3, and N3 had no significant reduction with aging (Figs. 1, 2, and 7). In particular, P3 was increased in CAB normal aging mice as age increased (Fig. 7E). In AD mice, P1 was also reduced comparing with WT and CBA mice (Figs. 3, 4, and 6) but not reduced with aging (Fig. 7A). Moreover, P3 in AD mice was diminished (Figs. 3, 4, and 6) and had no significant increase with aging (Fig. 7E). This is consistent with previous reports that AD patients had changes in amplitude of auditory evoked potentials (Buchwald et al., 1989; Irimajiri et al., 2005; Swords et al., 2018). In AD patients, cognitive N200 and P300 peaks (i.e., N2 and P3 peaks in mice) were reduced, different from the effect of normal aging (Morrison et al., 2018).

In mice, the latencies of peaks in AECP appeared reduced with normal aging (Fig. 2B). However, there were no significant delay of AECP peaks in AD mice except peak N2 at 1 year old (Fig. 5). Actually, the latencies of peak P1 and N1 in AD mice at 1-3 months old (Fig. 5B-D) and the latencies of N3 at 10-12 months old (Fig. 5F) were shorter than those in WT mice. This may arise from the fact that the neural interactions in AD mice are less than those in WT mice due to degeneration.

The auditory system has the advantage in comparison with other sensory systems. Decline in function over time in different nuclei (centers) can easily be repeatedly, quantitatively measured, and located by recordings of auditory evoked potentials. Indeed, our studies demonstrated that AECP and ABR recording (Liu et al. 2020) could serve as a simple, repeatable, noninvasive method to assess AD development and progression.

Acknowledgement: This work was supported by NIH R01 DC 017025 and DC 017025-01S1 to HBZ.

Author Contributions:

HBZ conceived the general framework of this study. ML, LML, KC and HBZ performed the experiments. ML, LML, and HBZ analyzed data. HBZ wrote the paper. All authors reviewed the manuscript and provided the input.

Conflict of Interest:

The authors declare no competing interests.

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Figure legends:

Fig. 1. Auditory evoked cortex potential (AECP) in CBA/CaJ mice at different ages. AECP was evoked by a click stimulus (85 dB SPL) in alternative polarity. Traces were averaged from the recorded mice with both genders. Error bars represents SEM. Mouse age is indicated in each panel. N is the mouse number. AECP in mice has three positive peaks, i.e., P1, P2, and P3, and three corresponding negative N1, N2, and N3 peaks. Red arrows indicate P3 peak that is clearly visible in AECP at 3 months old and afterward.

Fig. 2. Changes of AECP in normal aging CBA/CaJ mice. N is the mouse number. **A:** P1, P2, and P3 peak-times at the age of 6 months old. The red lines in boxes represent mean leaves. **B:** Changes of AECP peak-times in normal aging. The statistical t tests were performed by comparing peak-times at the defined age to those at age of 3 months old. **C-D**: Changes of peak-amplitudes with aging. The amplitudes of early waveforms P1, N1, and P2 in AECP decrease with aging, whereas the peak-amplitudes of later waveforms N2, P3, and N3 have no significant change with aging. The statistical analyses were performed by comparing peak-amplitudes at the defined age to those at age of 3 months old. *: $P<0.05$, **: $P<0.01$ (t test, two-tail).

Fig. 3. AECP traces recorded from APP/PS1 AD mice at different ages. WT littermates served as control. Red and black traces represent averaged AECP traces recorded from APP/PS1 AD mice and WT mice, respectively. Error bars represents SEM. Mouse age is indicated in each penal. N is the mouse number. Blue arrows indicate a notch at the

descending side of peak P2 in AD mice at 1-3 months old. Red arrows indicate the P3 peak clearly visible in WT mice but not in AD mice after 3 months old.

Fig. 4. Changes of AECP peaks in APP/PS1 AD mice with aging. WT littermates served as control. Peaks of AECP recorded from each mouse were measured and averaged. Peak name is presented at the top middle place in each panel. N is the mouse number. Panel E shows that the amplitudes of peak P3 in AD mice after 3 months old are significantly less than those in WT mice at the same age. *: $P<0.05$, **: $P<0.01$ (t test, two-tail).

Fig. 5. Changes of AECP peak-times in APP/PS1 AD mice with aging. Peak name is presented at the top middle place in each panel. N is the mouse number**.** The peak-times in AD mice were compared with those in WT littermates at the same age. *: $P<0.05$, **: $P<0.01$ (t test, two-tail).

Fig. 6. Comparison of AECP changes among CBA/CaJ mice, APP/PS1 AD mice, and WT littermate mice at different ages. Peak name is presented at the top middle place in each panel. Animal numbers in each group are presented within the bars in panel **A.** Panel **E** shows that peak P3 in the AD mice has significant reduction in comparison with those in CBA and WT littermate mice. *: $P<0.05$, **: $P<0.01$ one-way ANOVA with a Bonferroni correction.

Fig. 7. Comparison of the aging effects on AECP in CBA/CaJ, APP/PS1 AD, and WT littermate mice. The amplitudes of peaks were normalized to those at 3 months old in

each group. Peak name is presented at the top middle place in each panel. N is the animal number. The statistical t test with two-tail was performed by comparing to that at 3 months in each group. *: $P<0.05$, **: $P<0.01$.

Fig. 8. Expression of amyloid precursor protein (APP) in the auditory cortex (AC) in APP/PS1 AD mice. **A-C:** Immunofluorescent staining of brain slices for APP (red) in APP/PS1 AD mice and WT mice at different ages. Mice were 2 and 12 months old. AC: Auditory cortex. **D-E:** High-magnitude images of positive APP-labeling in the AC area. Positive labeling for APP is visible at the AC area in the APP/PS1 AD mouse at 2 month old. White arrows in panel D indicate APP deposits in the AC presented in the typical form of plaques in the 12 months old mouse. Scale bar: 100 μm in **A-C**, 10 μm in **D-E**.

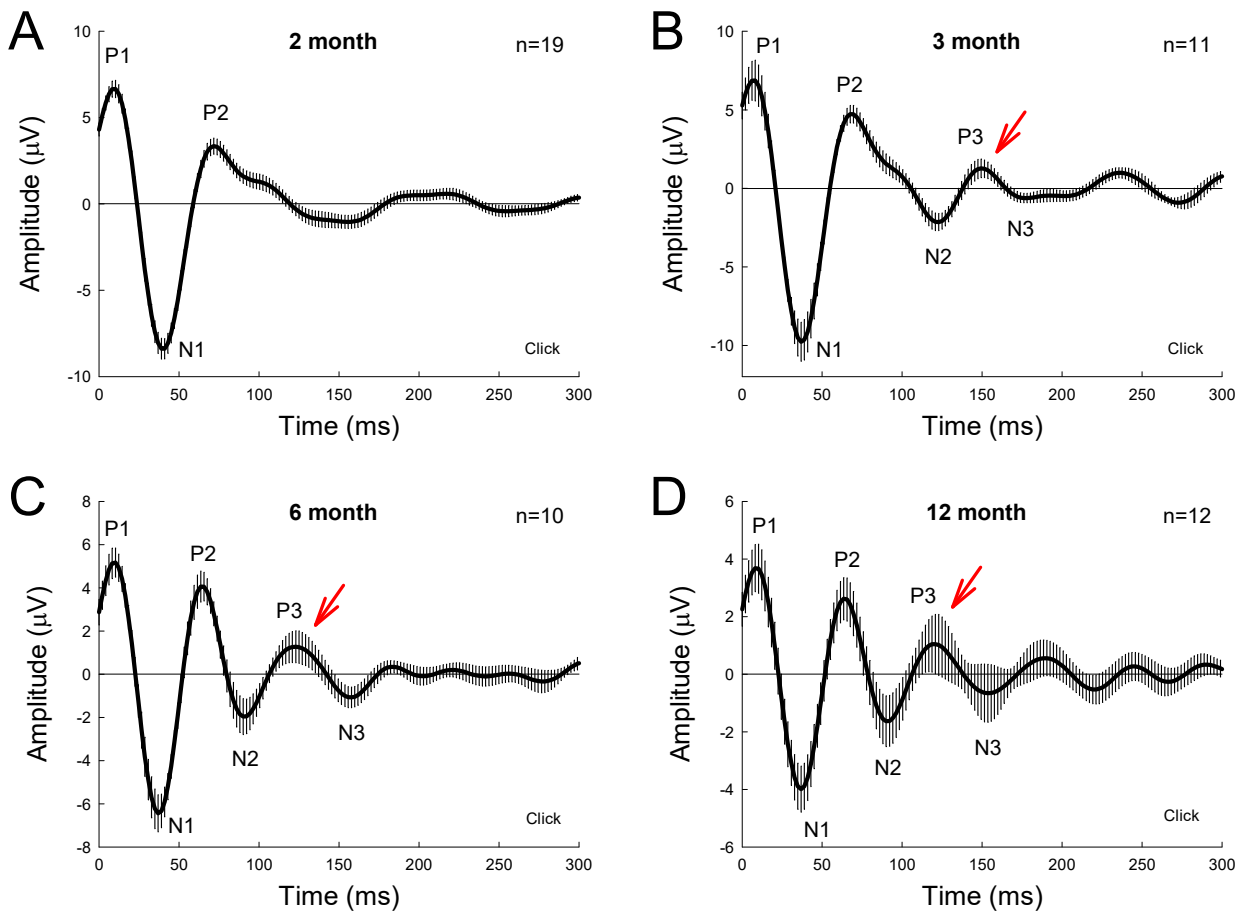

Fig. 1. Auditory evoked cortex potential (AECP) in CBA/CaJ mice at different ages.

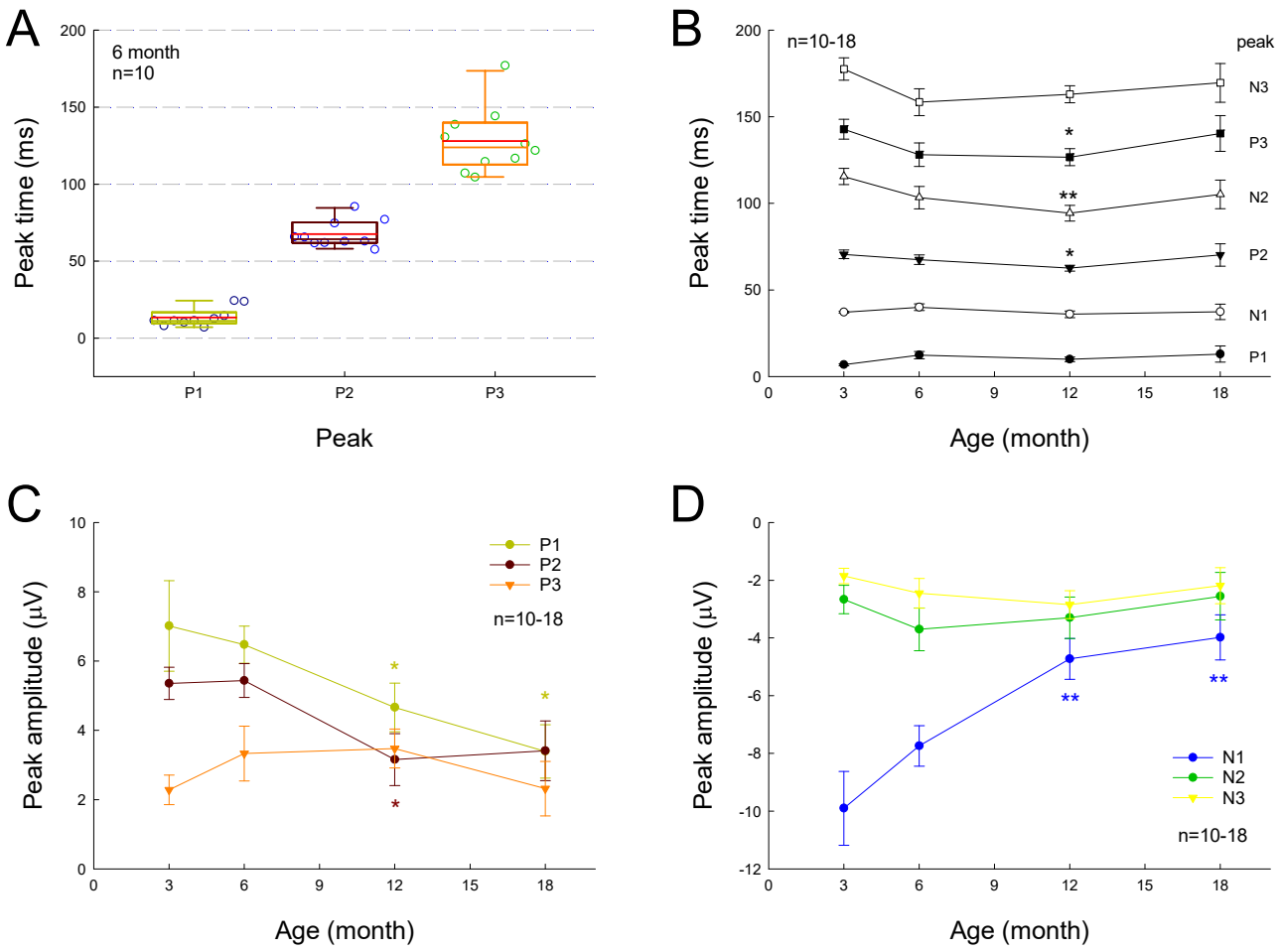

Fig. 2. Changes of AECP in normal aging CBA/CaJ mice.

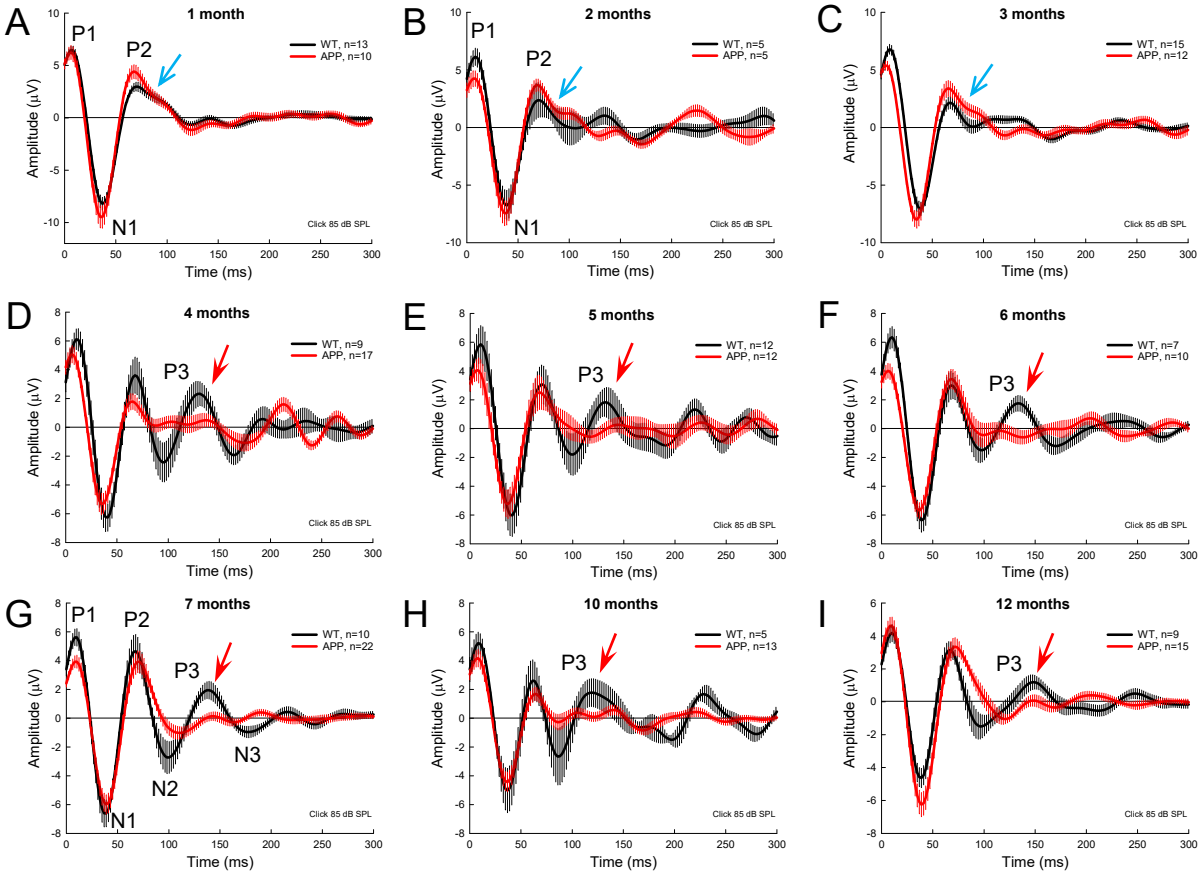

Fig. 3. AECP traces recorded from APP/PS1 AD mice at different ages.

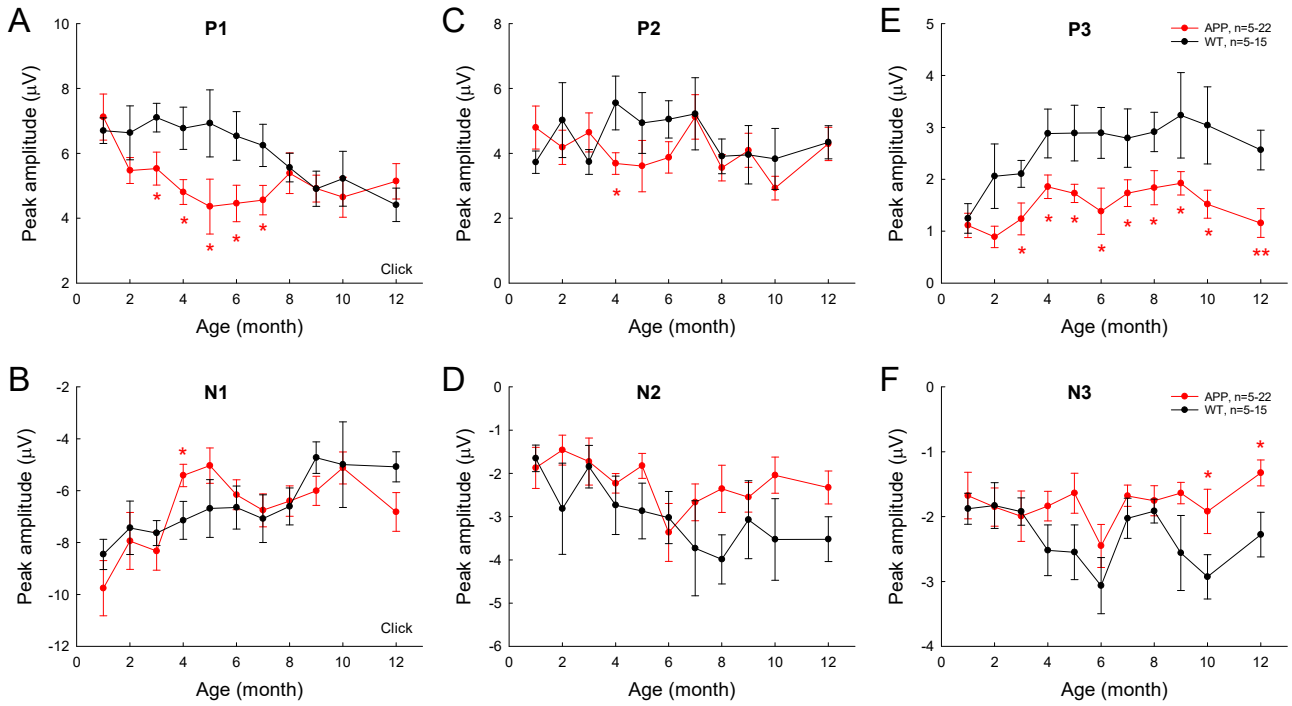

Fig. 4. Changes of AECP peaks in APP/PS1 AD mice with aging.

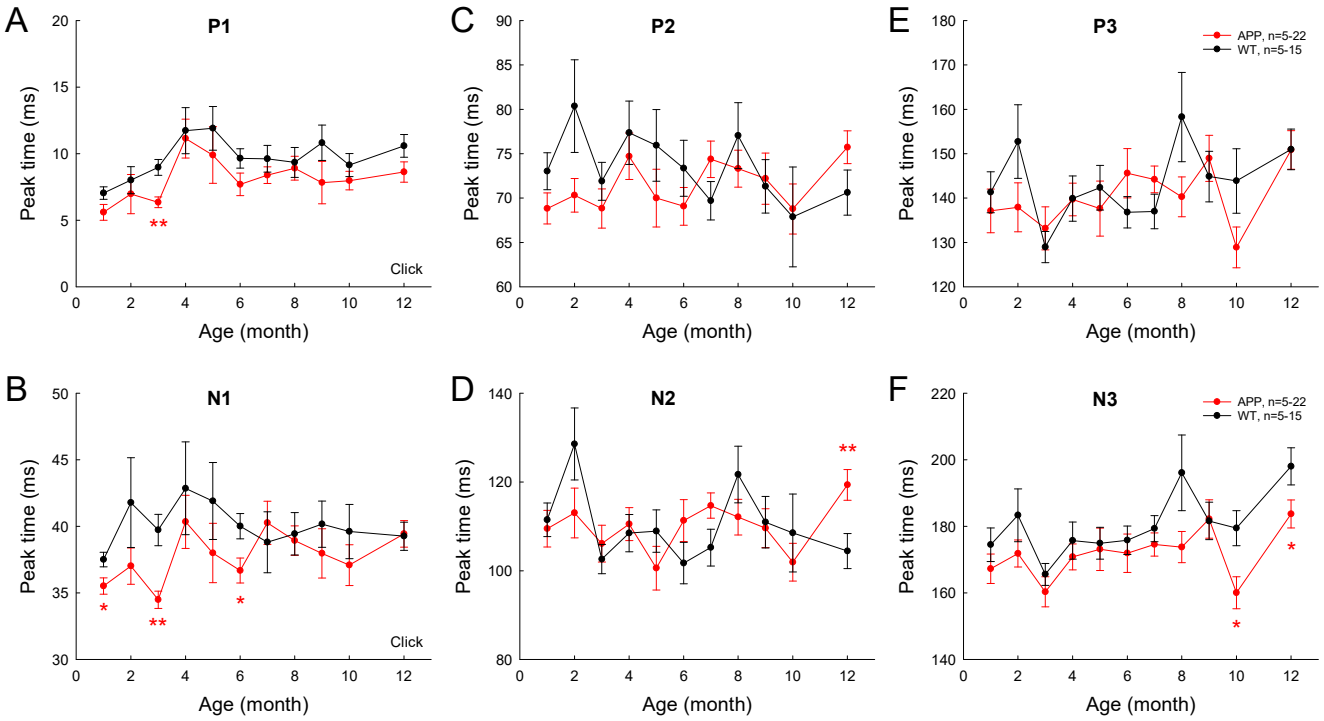

Fig. 5. Changes of peak-times of AECP in APP/PS1 AD mice with aging.

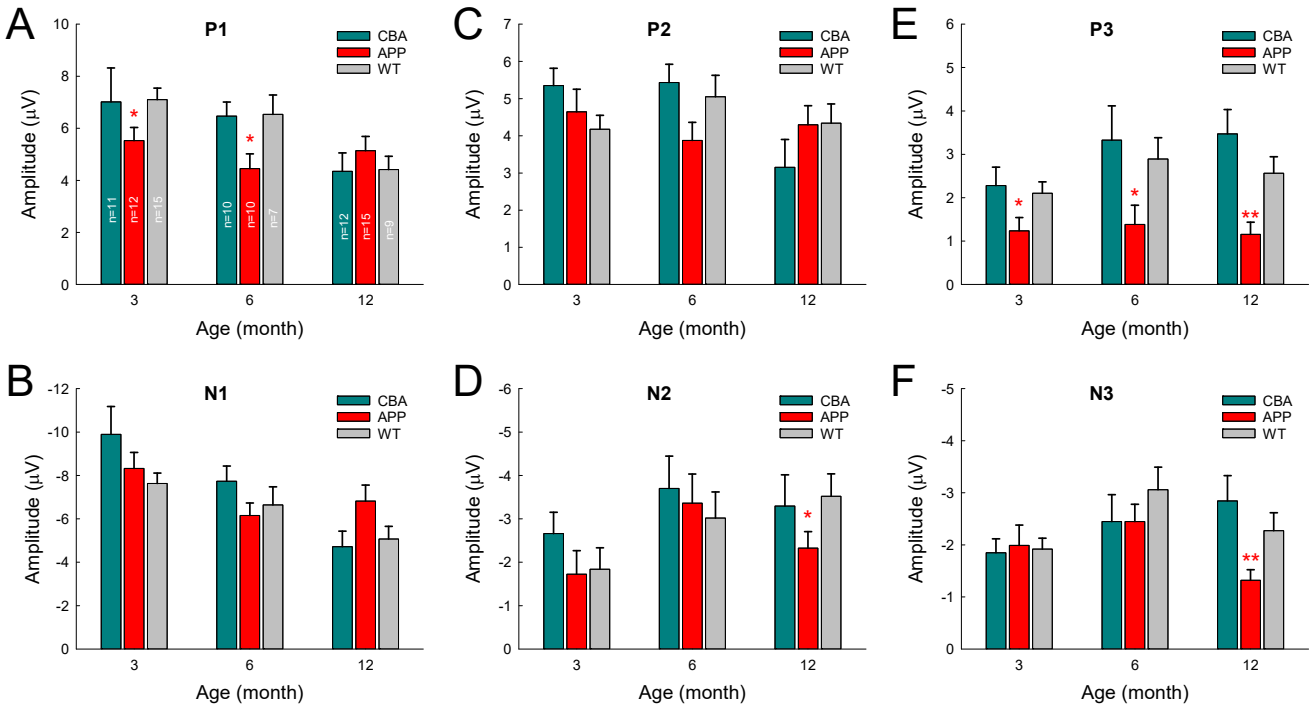

Fig. 6. Comparison of AECP peaks among CBA/CaJ mice, APP/PS1 AD mice, and WT littermate mice at different ages.

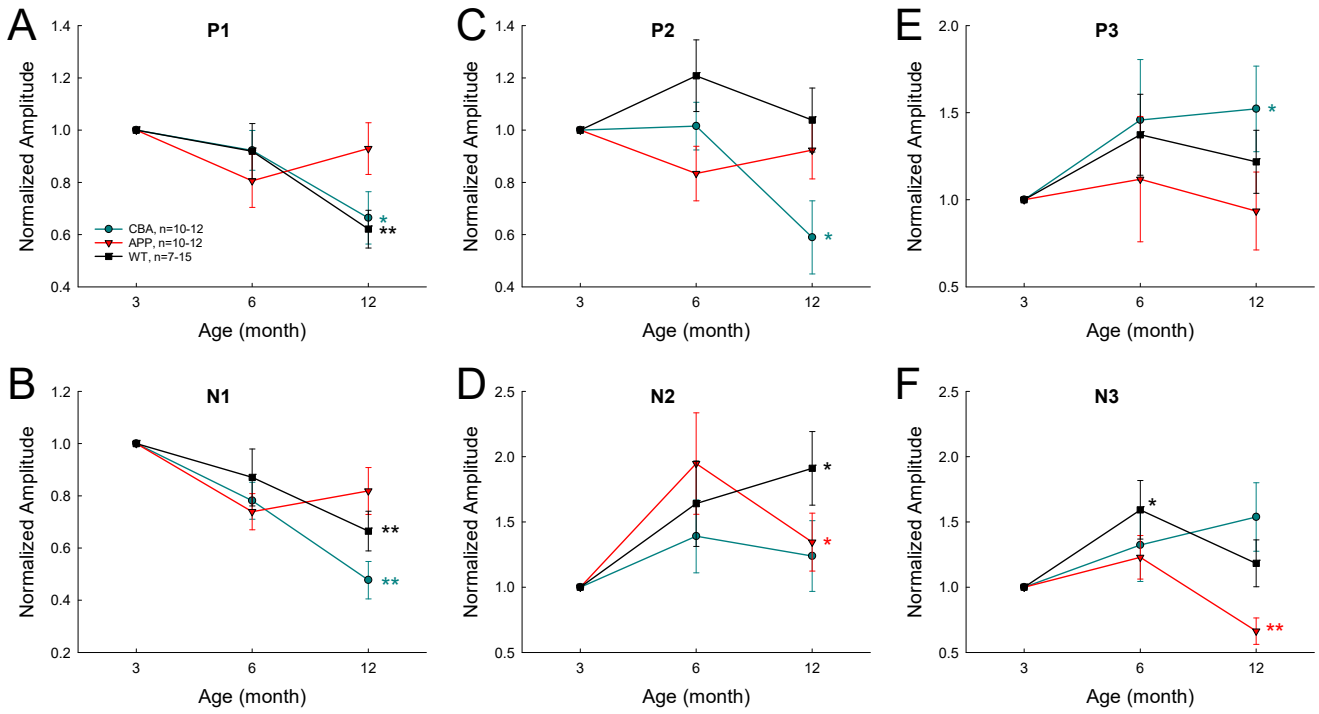

Fig. 7. Changes of the aging effects on AECP in CBA/CaJ, APP/PS1 AD, and WT littermate mice.

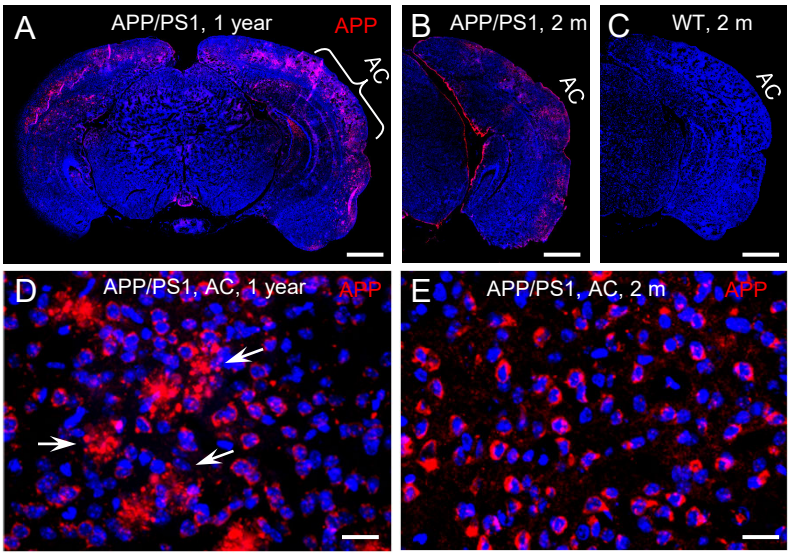

Fig. 8. Expression of amyloid precursor protein (APP) in the auditory cortex (AC) in APP/PS1 AD mice.