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Hearing loss as a risk factor for cognitive impairment and loss of synapses in the hippocampus

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ABSTRACT

Although epidemiological studies have identified an association between hearing loss and cognitive impairment, there is a lack of biological evidence detailing the mechanisms underlying this association. The present study investigated the effects of hearing loss on cognitive impairment using an at-risk model. In this animal model, amyloid-β (Aβ) was administered to the brain to such an extent that it did not cause cognitive impairments but made the brain vulnerable to risk factors. This study included four experimental groups based on hearing level and Aβ administration. Behavioral tests were conducted to evaluate cognitive function, and synaptic protein levels were measured in the hippocampus and prefrontal cortex. The group with hearing loss and Aβ administration showed significantly greater deficits on cognitive tests associated with the hippocampus than the other three groups (only Aβ administration, only hearing loss, and without hearing loss or Aβ administration). The hearing loss and Aβ administration group also had significantly lower levels of synaptic proteins in the hippocampus than the other groups. The present results suggest that hearing loss may act as a risk factor for cognitive impairment in Alzheimer's disease. Additionally, the present findings indicate hearing loss may cause hippocampal synapses to be more vulnerable to Aβ-induced damage.

1. Introduction

In 2016, approximately 43.8 million people suffered from dementia worldwide. Furthermore, the worldwide death rate associated with dementia was 2.4 million people, which made it the fifth leading cause of death [1]. The leading cause of dementia is Alzheimer's disease (AD) [2] and, therefore, there is an urgent need for the development of treatments for AD. Although much research has been conducted in this area, the currently available treatments for AD have yet to achieve significant clinical efficacy in that they can partially stabilize the symptoms of this disease but not correct it [3].

It is also important to identify risk factors for AD, as this information will allow us to develop methods preventing AD development or slowing disease progression. Age, family history, and heredity are the most important risk factors of AD [4] and can be used to predict its occurrence. However, these factors cannot be modified and, thus, cannot contribute to the prevention of AD. Recent epidemiological evidence suggests that there is an association between hearing loss and cognitive impairment [5–8] and other studies have shown that hearing loss may be a potentially modifiable risk factor of AD [9]. Approximately one-third of elderly people 65 years of age and older have hearing loss, which can be ameliorated by hearing aids and cochlear implants. Therefore, if hearing loss is a risk factor of cognitive impairment and its mechanisms can be identified, then the treatment of hearing loss can contribute to the prevention of AD. However, the causal relationship between hearing loss and AD remains controversial. For example, it has been suggested that the association between hearing loss and AD exists due to difficulties in cognitive function tests that patients with hearing loss experience due to poor verbal communication. Furthermore, the biological mechanisms that underlie this association have yet to be elucidated.

Thus, the present study employed animal models to investigate

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; OPT, object-in-place task; OLT, object location task; NOR, novel object recognition task; ABR, auditory brainstem response; NH-SA, normal hearing-subthreshold amyloid-β; deaf-SA, deaf-subthreshold amyloid-β; NH-NA, normal hearing-non amyloid-β; deaf-NA, deaf-non amyloid-β

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whether hearing loss would be a risk factor for AD and to assess the mechanisms by which hearing loss may act as a risk factor. Because several empirical cases and other evidence indicates that hearing loss alone does not lead to cognitive impairment [10], a subthreshold amyloid-β (Aβ) model of AD [11] was used in the present study. In this model, Aβ is administered to the brain to such an extent that it does not cause cognitive impairments but makes the brain vulnerable to risk factors so that it might be possible to verify whether hearing loss would be a risk factor for cognitive impairment.

2. Methods

2.1. Experimental design

This study was approved by the Institutional Animal Care and Use Committee of Chung-Ang University (2016-00086) and Seoul National University Hospital (16-0133-C1A0) and all experiments were conducted in accordance with relevant guidelines and regulations. Seven-week-old male Wistar rats (200–250 g) were used and all animals were adapted to laboratory conditions for 1 week prior to the start of the experiment and housed in a temperature- and humidity-controlled room with a 12-h light:dark cycle with food and water available *ad libitum*. Auditory brainstem response (ABR) recordings and surgical procedures were performed under anesthesia induced by the intraperitoneal administration of ketamine hydrochloride (100 mg/kg; Ketamine®, Yuhan Co.; Seoul, Korea) mixed with xylazine (10 mg/kg; Rompun®, Bayer-Korea; Seoul, Korea).

The present study consisted of two stages: determining the time course of cognitive decline following hearing loss and then evaluating changes in cognitive function and synaptic protein levels after induction of the hearing loss (Fig. 1). In the first stage, 10 rats were randomly divided into two groups: a pilot-normal hearing-subthreshold Aβ group (pilot-NH-SA; n = 5) that underwent a sham surgery and the infusion of subthreshold Aβ and a pilot-deaf-subthreshold Aβ group (pilot-deaf-SA; n = 5) that underwent bilateral cochlear ablation and infusion of subthreshold Aβ. The infusion of subthreshold Aβ for 2 weeks began 3 weeks after surgery and the Y-maze test was performed every 2 weeks in all rats starting at 7 weeks after the surgery. The results of the first stage were used to determine the timepoints at which hearing loss induced a significant effect on cognitive impairment.

In the second stage, 26 rats were randomly divided into four experimental groups: a normal hearing-non Aβ group (NH-NA; n = 6) that underwent a sham surgery but not infusion of subthreshold Aβ, a normal hearing-subthreshold Aβ group (NH-SA; n = 6) that underwent a sham surgery and the infusion of subthreshold Aβ, a deaf-non Aβ group (deaf-NA; n = 7) that underwent bilateral cochlear ablation but not infusion of subthreshold Aβ, and a deaf-subthreshold Aβ group (deaf-SA; n = 7) that underwent bilateral cochlear ablation and the infusion of subthreshold Aβ. The infusion of subthreshold Aβ for two weeks began 9 weeks after surgery and cognitive tests including the Y-maze test, object-in-place task (OPT), object location task (OLT), and novel object recognition task (NOR), were performed to all rats 11 weeks after surgery. After the cognitive function tests, tissue samples were harvested from the hippocampus and prefrontal cortex. One animal in the deaf-SA group exhibited postural asymmetry when picked up after the bilateral cochlear ablation and was excluded from the experiment. During the breeding period, one animal in the NH-SA group and one animal in the deaf-SA group died. Ultimately, the NH-NA, NH-SA, deaf-NA, and deaf-SA groups consisted of 6, 5, 7, and 5 animals, respectively. We performed an additional experiment using another nine rats to assess whether the animals preferred familiar or novel objects in the NOR.

2.2. ABR recordings

ABR recordings were conducted in all rats before surgery and 1 week, 6 weeks, and 11 weeks after surgery to measure hearing levels. ABRs on the left side were recorded with subdermal needle electrodes between the left mastoid and the nape of the neck with the right mastoid as the return while ABRs on the right side were measured by reversing the direction of the electrodes. ABRs were recorded with high-frequency transducers (HFT9911–20–0035) and software (ver. 2.33)

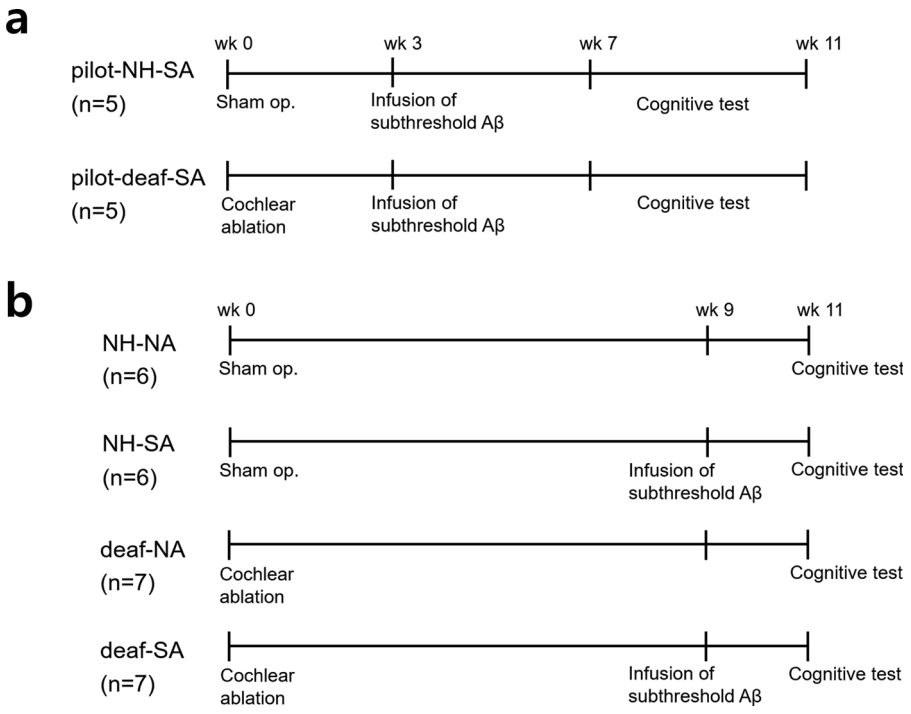

Fig. 1. Experimental flow of the first (a) and second (b) stage.
Aβ, amyloid-β; NH-SA, normal hearing-subthreshold amyloid-β; deaf-SA, deaf-subthreshold amyloid-β; NH-NA, normal hearing-non amyloid-β; deaf-NA, deaf-non amyloid-β.

from SmartEP (Intelligent Hearing Systems; Glenvar Heights, FL, USA) and the responses were amplified (100,000×), band pass-filtered (100–1500 Hz), and averaged over 512 stimulus repetitions. Tone pips of 8, 16, and 32 kHz were used as sound stimuli (5-ms duration, cos shaping, 21 Hz) and stimulus intensity was reduced in 5 dB SPL decrements. Two researchers, blind to the experimental conditions, determined the lowest stimulus intensity that evoked a recognizable response, and that was regarded as the threshold.

2.3. Cochlear ablation

Cochlear ablation was performed on both sides as previously described [12]. Briefly, after a retroauricular incision, the external auditory canal was opened and the tympanic membrane and ossicles, except for the stapes, were removed. Then, a small hole was made on the bony wall of the cochlea and the contents of the cochlea were ablated with a dental pick. A small amount of soft tissue was packed into the small hole on the bony wall of the cochlea. In the sham surgery, the same operative procedure was performed before the point of opening the external auditory canal.

2.4. Behavioral tests for vestibular deficits

To exclude the effects of vestibular function deterioration during cochlear ablation, the behavioral test for vestibular deficits was performed the day and week after surgery as previously described [13]. Briefly, the behavioral scoring for vestibular deficits consisted of three components: postural asymmetry, head roll tilt, and nystagmus (Table S1). If any deficits were found in any of these three components, the animal was excluded from the experiment.

2.5. Infusion of subthreshold Aβ

The Aβ peptide solution was continuously administered into the intracerebroventricular space (160 pmol/day) for 2 weeks using a brain infusion cannula (Brain Infusion Kit 2, Alzet; Cupertino, CA, USA) that was connected to a mini-osmotic pump (Osmotic Pump 2002, Alzet). The infusion cannula was implanted into the right cerebral lateral ventricle (AP: −0.3, L: 1.2, V: 4.5) according to the coordinates of Paxinos and Watson (2006) [14]. The composition of the Aβ peptide solution, which does not induce cognitive impairment, has been described previously [11]. Briefly, a Aβ1-42 peptide solution (AnaSpec Inc.; San Jose, CA, USA) was dissolved in 35% acetonitrile/0.1% trifluoroacetic acid. The mini-osmotic pump was removed 2 weeks after implantation, and the remaining volume of Aβ1-42 peptide solution measured to confirm that the expected volume had been delivered; we subtracted the residual from the initial volume.

2.6. Cognitive testing

2.6.1. Y-maze test

Cognitive function was assessed by recording spontaneous alternation behavior in a single session in the Y-maze; the protocol for this task has been previously reported [15]. Briefly, each arm of the maze was 40 cm long, 30 cm high, and 15 cm wide and converged in a central triangle area. None of the animals had ever experienced a Y-maze before. All arms were brushed with 10% ethanol prior to each session to remove the possible effects of odor cues and the experimenter was not in the room during testing. Each rat was placed on one arm tip of the Y-maze and then allowed to walk around the maze for 7 min without restriction. Each session in the Y-maze was video recorded and analyzed later. The rat was considered to have entered the arm when its hind paws entered the arm and alternation was defined as successive entries into three arms based on overlapping triplets. The alternation percentage was calculated as follows: actual alternations / possible alternations (total number of arm entries minus two).

2.6.2. OPT, OLT, and NOR

The OPT, OLT, and NOR were conducted by modifying a previously reported method [16]. Beginning 4 days before the tests, the rats were placed in an open field box (58 × 42 × 35 cm) without stimuli for 10–15 min daily. Each session consisted of familiarization and test phases and either the type or location of the stimulus objects in the test phase was different from that in the familiarization phase. In the familiarization phase, the rats explored stimulus objects in the open field box for 5 min and were then returned to their home cage for a fixed amount of time (5 min for OPT and OLT and 3 h for NOR). Then, the rats were placed in the box again and allowed to explore the stimulus objects during the test phase. The experiment was video recorded in a room without the experimenter and the recorded video was analyzed later. Exploratory behavior was defined as directing the nose toward an object at a distance of less than 2 cm or touching the object with the nose or paws. A discrimination ratio was calculated as follows: (exploration time with the changed object - exploration time with the unchanged object) / (total exploration time with the changed and unchanged object). When exploration time was shorter than 15 s during the familiarization phase or shorter than 10 s in the test phase, the data were excluded from the analysis.

The test conditions are shown in Fig. S1. For the OPT familiarization phase, four different stimulus objects were placed in the corners of the box (10 cm from the wall). During the OPT test phase, the positions of two of the objects (which were both on the left or right of the box) were switched. For the OLT familiarization phase, two identical objects were placed in the corners of the box. During the OLT test phase, one object was repositioned to the corner adjacent to its original position; thus, the two objects were diagonal to each other. For the NOR familiarization phase, two identical objects were placed in the corners of the box. During the NOR test phase, one object was changed to a novel object. Before the NOR, we performed an additional experiment to assess object bias. After adaptation to the open field box, another nine rats explored the two objects (a familiar and a novel object) to be used in the NOR test phase for 5 min. The durations of time spent exploring each object were measured and compared.

2.7. Western blot analysis

After completion of the behavioral tests, all animals were euthanized and brain tissue samples were harvested from the hippocampus and prefrontal cortex based on the coordinates of Paxinos and Watson (2006) [14]. For the Western blot analyses, tissues from the hippocampus and prefrontal cortex of all groups were lysed in a radioimmunoprecipitation assay buffer (RIPA) buffer (iNtRON Biotechnology; Seoul, Korea) containing a protease inhibitor cocktail (Sigma; St. Louis, MO, USA), protein phosphatase inhibitor cocktail (AG Scientific; San Diego, CA, USA), and phenyl-methylsulfonyl fluoride (PMSF; Sigma). Then, the brain lysates were sonicated to ensure thorough lysis. The concentrations of the protein lysates were determined with a BCA assay and an identical amount of protein from each sample was electrophoretically separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in 4–12% Bis–Tris gels and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in 5% non-fat dry milk in Tris-buffered saline (TBS) and 0.1% Tween-20 (TBS-T) and then incubated with the following primary antibodies at 4°C overnight: postsynaptic density protein 95 (PSD95; ab18258, Abcam; Cambridge, UK), synaptophysin (mab268, Millipore; Burlington, MA, USA), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII; ab52476, Abcam), phosphorylated CAMKII (pCaMKII; 3361 s, Cell Signaling Technology; Danvers, MA, USA), N-methyl D-aspartate receptor subtype 2B (NR2B; 06–600, Millipore), and α-tubulin (05–829, Millipore). Next, the membranes were washed with TBS-T for 30 min and incubated with secondary IgG-HP antibodies against each primary antibody for 1 h. Then, the membranes were washed with TBS-T and incubated with an ECL chemiluminescent

reagent. Finally, peroxidase activity was detected with LAS 4000 (GE Healthcare Life Science; Marlborough, MA, USA); the optical densities were normalized with a standard protein.

2.8. Statistical analyses

IBM SPSS software version 21.0 (IBM; New York, NY, USA) was used for all statistical analyses. ABR thresholds were analyzed with one-way analysis of variance (ANOVA) tests. Scores on the cognitive tests in the first stage of the experiment were analyzed with repeated measures ANOVA tests and scores at each timepoint were analyzed with unpaired two-tailed Student's *t*-test. Scores on the cognitive tests in the second stage of the experiment were analyzed with one-way ANOVAs and paired t-tests. The results of the Western blot analyses were analyzed with one-way ANOVAs. All post hoc testing was performed using Tukey's tests.

3. Results

3.1. ABR recordings

Prior to surgery, the baseline ABR thresholds at 8, 16, and 32 kHz ranged from 20 to 35 dB SPL in all animals; these values did not differ significantly among the groups ($p > 0.05$). At 1 week, 6 weeks, and 11 weeks after surgery, the ABR thresholds at 8, 16, and 32 kHz ranged from 20 to 35 dB SPL in the NH group but were higher than 80 dB SPL in the deaf group (Fig. 2).

3.2. Dose of Aβ1-42 peptide solution delivered

The daily volumes of delivered Aβ1-42 peptide solution ranged from 12.0 to 11.9 μL, corresponding to 161.0 to 159.0 pmoL/day of the Aβ1-42 peptide, similar to the anticipated volumes. The daily doses did not differ significantly between the groups ($p > 0.05$).

3.3. Time course of cognitive decline following hearing loss

In the first stage of the experiment, the time course of cognitive decline following hearing loss was evaluated using the results of the Y-maze test (Fig. 3a). The influence of hearing loss was explored with a repeated measures ANOVA using the Y-maze scores across time as a repeated measure (7, 9, and 11 weeks after surgery) and the groups as fixed factors. Mauchly's test of sphericity indicated that the assumption

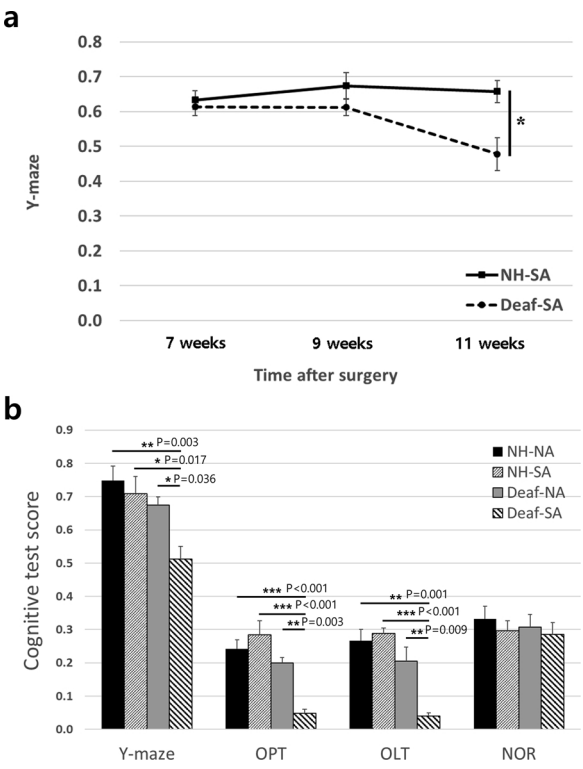

Fig. 3. Cognitive test results. (a) Time course of cognitive decline following hearing loss. Y-maze scores were significantly lower in the pilot-deaf-SA group compared to the pilot-NH-SA group at 11 weeks after surgery. (b) In the Y-maze, OPT, and OLT tests, the deaf-SA group had significantly lower scores than the other three groups in the second stage of the experiment. All data are presented as a mean ± SEM. (a) Unpaired two-tailed Student's *t*-test at each timepoint. (b, c) One-way ANOVA followed by Tukey's post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001.
SA, sub-amyloid-β; NH, normal hearing; OPT, object-in-place task; OLT, object location task; NA, non-amyloid-β.

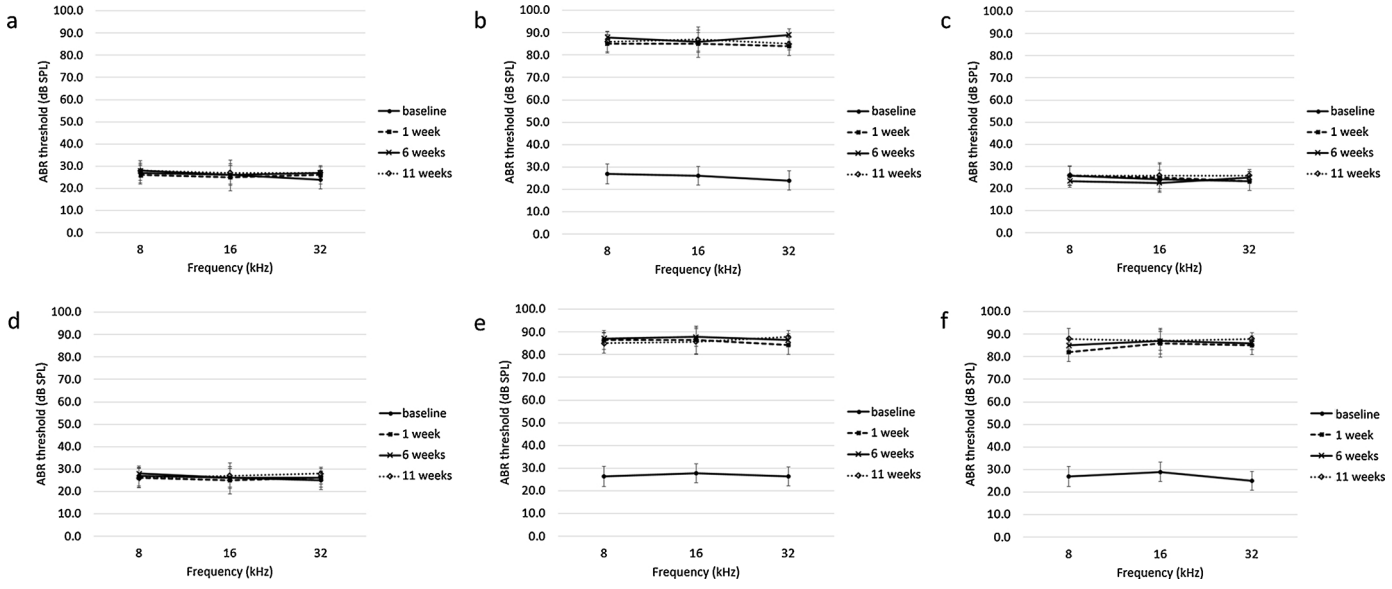

Fig. 2. ABR thresholds before surgery and 1 week, 6 weeks, and 11 weeks after surgery. (a) pilot-NH-SA group. (b) pilot-deaf-SA group. (c) NH-NA group. (d) NH-SA group. (e) deaf-NA group. (f) deaf-SA group. Error bars indicate standard deviation.
ABR, auditory brainstem response; SPL, sound pressure level; NH, normal hearing; SA, sub-amyloid-β; NA, non-amyloid-β.

of sphericity for time had been violated ($p = 0.453$) and, therefore, the results for time are reported using the Greenhouse-Geisser correction ($\varepsilon = 0.832$). The Y-maze scores changed over time ($p = 0.046$) and there was a significant interaction between time and group ($p = 0.032$); thus, the main effects for group are reported at each timepoint. The Y-maze scores of the pilot-NH-SA and pilot-deaf-SA groups did not significantly differ at 7 or 9 weeks after surgery ($p = 0.624$ and $p = 0.208$, respectively) but the Y-maze scores of the pilot-deaf-SA group were significantly lower than those of the pilot-NH-SA group at 11 weeks after surgery ($p = 0.014$).

3.4. Cognitive function and synaptic maker protein levels after hearing loss

The cognitive testing results in the second stage of the experiment are displayed in Fig. 3b and Table S2 and S3. The time spent by the animals in exploration exceeded 15 s during the familiarization phases and 10 s during the test phases of the OPT, OLT, and NOR. No animal was excluded from the analysis. The total time spent exploring objects during the familiarization and test phases of the OPT, OLT, and NOR did not differ among the groups (Table S2). During the familiarization phases of the OPT and OLT, no significant differences in the time spent exploring objects that were switched and those not switched during the test phases were apparent (Table S3). This was also the case for the additional experiment of the NOR (21.8 ± 3.3 and 21.6 ± 4.0 s respectively, $p = 0.852$, paired t-test).

In the Y-maze, OPT, and OLT tests, the deaf-SA group had significantly lower scores than the other three groups ($p < 0.05$, Fig. 3b). There were no significant differences among the other three groups on those three tests and no significant differences among all four groups in the NOR test.

The present study also investigated molecular changes in the hippocampus and prefrontal cortex of all groups by quantifying synaptic protein levels with Western blot analyses. In the hippocampus, there were significant decreases in NR2B and PSD95, which are post-synaptic markers, and synaptophysin, which is a pre-synaptic marker, levels in the deaf-SA group (Figs. 4a–d and S2) but no significant changes in the other three groups. Additionally, there were no significant changes in the phosphorylation levels of CaMKII (Fig. 4a and e). In the prefrontal cortex, PSD95 levels significantly decreased in the deaf-SA group compared to the NH-NA and deaf-NA groups (Fig. 4f and i). Synaptophysin levels significantly decreased in the NH-SA and deaf-SA groups compared to the NH-NA and deaf-NA group showed decreasing trends (Fig. 4f and h). The phosphorylation levels of CaMKII decreased in all other groups compared to the NH-NA group (Fig. 4f and j). NR2B levels in the prefrontal cortex did not significantly differ among the groups (Fig. 4f and g).

4. Discussion

Although several epidemiological studies have suggested that hearing loss is a risk factor for cognitive decline [6–8,17], the underlying mechanisms remain unclear. Three representative hypotheses have been presented; they involve the effects of hearing impairments on cognitive load and brain structure and decreased social engagement [18]. The cognitive load hypothesis suggests that auditory perceptual processing requires more cognitive resources when the auditory signal is degraded, which results in the degradation of other cognitive processes, such as working memory. Another hypothesis proposes that impaired auditory signals and reduced stimulation from an impaired cochlea cause changes in brain structure. This would make the brain more vulnerable to brain pathology-causing factors, such as Aβ accumulation, neurofibrillary tangles, and microvascular disease, and lead to an increased risk of dementia. The third hypothesis suggests that cognitive function is degraded by social isolation due to hearing loss. However, few studies have provided evidence supporting these hypotheses. Thus, the present study attempted to determine whether hearing loss would act as a risk factor for AD and to identify the mechanisms underlying this association.

When planning the present experiments, it was important to consider that cognitive dysfunction will not be induced when only hearing loss is present. The results of a follow-up study investigating cognitive function in subjects who developed hearing loss in childhood reported that long-term sensory impairment alone has a negligible effect on one's overall level of cognitive function [10]. Therefore, the present study compared cognitive function in animals with hearing loss and normal hearing using a model of subthreshold Aβ, which has been published under the name of the at-risk model [11]. This model is intended to represent individuals with a predisposition for Aβ buildup but normal cognitive function. Thus, it is possible to investigate whether certain factors may be risk factors of AD.

In the present study, four experimental groups based on hearing level and the subthreshold administration of Aβ were formed and cognitive tests known to be related to specific brain regions were conducted. Cognitive tests associated with the hippocampus, such as the Y-maze, OPT, and OLT [16], revealed significant decreases in cognitive function in the deaf-SA group after hearing loss, as compared to the other groups. However, there were no significant differences among the groups in the NOR. The hippocampus may affect NOR results when the time between the familiarization and test phases is extended [19,20]. However, others have reported that the hippocampus does not influence NOR results regardless of the time interval between the two phases [16,21–24]. The discrepancies may be attributable to differences in the methods used to eliminate hippocampal function and the experimental conditions under which NOR was performed. A study that evaluated NOR exactly as we did reported that the hippocampus did not affect the results [16]. Therefore, in our experiment, the hippocampus may not affect NOR results. Taken together, these results suggest that hearing loss affected the hippocampus and may be a risk factor for cognitive impairment.

Comparisons of synaptic protein levels in the hippocampus between the NH-NA and NH-SA groups revealed no significant differences. These results indicate that the subthreshold administration of Aβ did not affect synaptic protein levels in the hippocampus in normal hearing animals. The changes in synaptic protein levels in the hippocampus after hearing loss mirrored the results of the cognitive testing: the deaf-SA group exhibited a significant decrease in synaptic proteins compared to the other three groups. These data indicate that cognitive impairment may be accelerated by the synergistic effects of hearing loss and Aβ due to synaptic loss. In the case of prefrontal cortical synaptic protein levels, some proteins in the deaf-SA group exhibited a reduction but these changes were not consistent and were not likely to be affected by hearing loss.

The present study demonstrated that hearing loss might act as a risk factor for cognitive impairment in AD patients and that hearing loss may cause hippocampal synapses to be more vulnerable to brain pathology. This finding indicates that there are connections between the central auditory pathway and the hippocampus, which has been proposed in previous studies. For example, there are changes in the hippocampus following sound exposure [25–29] and the use of anterograde tracers revealed that the hippocampus receives signals from the auditory cortex via the entorhinal cortex [30]. Therefore, degeneration in the central auditory pathway induced by hearing loss [31,32] may cause the degeneration of hippocampal synapses or make these synapses more vulnerable to damage. This hypothesis is supported by findings showing that focal cortical infarction of brain regions that are remote but connected to the hippocampus induce neuronal loss in the hippocampus [33]. Further studies are needed to obtain solid conclusions.

The present study has several limitations that should be noted. First, the development of hearing loss and Aβ deposition in the animal models used in this study differ from those in actual humans. In most humans, hearing loss and Aβ deposition progress slowly and, therefore,

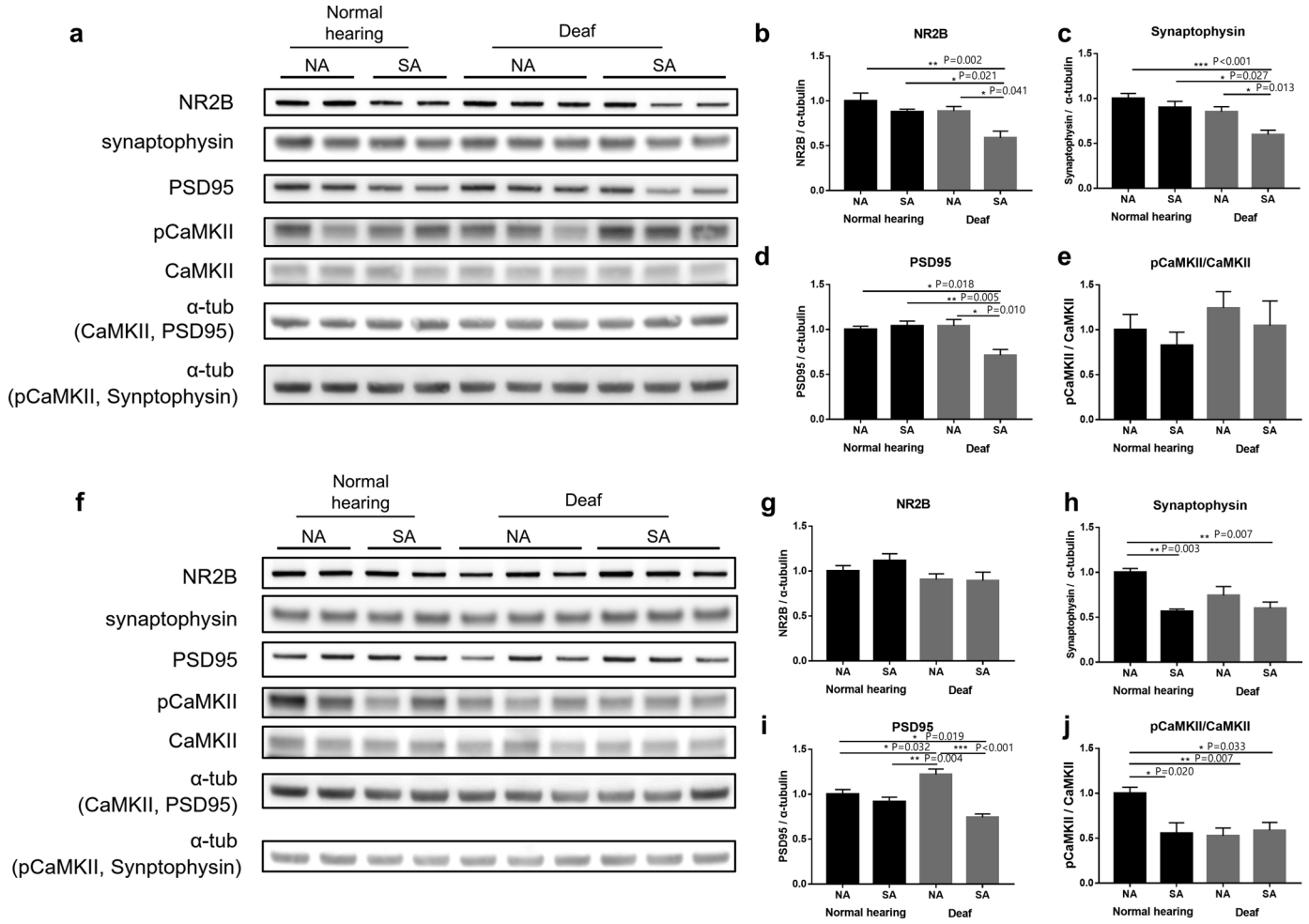

Fig. 4. Synaptic marker proteins are altered by hearing loss and Aβ infusion in the rat brain. Pre- and post-synaptic marker protein levels in the hippocampus decreased following hearing loss and Aβ infusion. (a) Representative images and (b–e) quantificational graphs (n = 5–7). Some pre- and post-synaptic marker protein levels in the prefrontal cortex decreased following hearing loss and Aβ infusion. (f) Representative images and (g–j) quantificational graphs (n = 5–7). All data are presented as a mean ± SEM. One-way ANOVA followed by Tukey's post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001.
NA, non-amyloid-β; SA, sub-amyloid-β; Aβ, amyloid-β.

it will be necessary to develop a novel animal model in which hearing loss and Aβ deposition progress in a manner similar to that of humans. Second, the present study showed that there was a decrease in hippocampal synapses following hearing loss. However, the locations and roles of the degenerated synapses could not be identified and further research will be necessary to clarify these findings.

5. Conclusions

The present study showed that hearing loss may act as a risk factor for cognitive impairment in AD. Furthermore, hearing loss may make synapses in the hippocampus more vulnerable to damage that can result in brain pathology.

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