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Abstract: Purpose: Accumulation of amyloid beta (A□) is thought to be the
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determine possible changes relevant to the accumulation of Aβ1-42.
Materials and Methods: In this study, rats were randomized to following
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Aβ-2(2µg/µl), Aβ-3(3µg/µl), Aβ-4(4µg/µl), Aβ-5(6µg/µl), Aβ-6(8µg/µl) and
Aβ-7(10µg/µl) groups obtained by injection of 5 µl per ventricle. Then,
AEPs were recorded in freely-moving rats. Latencies and amplitudes of
AEPs, evoked power, inter-trial phase synchronization, and auditory
evoked gamma responses were obtained in response to auditory stimulus.
Furthermore, Aβ1-42 levels were determined in the temporal cortex.
Results: Aβ1-42 levels were significantly higher in the temporal cortex
in Aβ groups compared to the Sham. In frontal and parietal regions, P1N1
amplitudes were significantly decreased in A□-3, 4, 5 and 6 groups, and
N1P2 amplitudes were significantly decreased in all A□ groups, whereas in
temporal regions, P1N1 and N1P2 amplitudes were decreased in A□-2,
3,4,5,6 and 7 compared to the Sham. In the evoked gamma power and phase
synchronization of gamma responses, we detected significant decrement
after A□-4 group, whereas a significant decrement in the filtered gamma
responses was observed in Aβ groups compared to the Sham.
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Title: Auditory evoked potentials might have the potential to serve as early indicators related to amyloid beta peptide toxicity

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Abstract

Purpose: Accumulation of amyloid beta (Aβ) is thought to be the major cause of the development and progression of Alzheimer's disease (AD). The aim of this study is to elucidate the effects of $A\beta_{1-42}$ at increasing concentrations on auditory evoked potentials (AEPs) and to determine possible changes relevant to the accumulation of $A\beta_{1-42}$.

Materials and Methods: In this study, rats were randomized to following groups (n= 10 rats per each group): Sham (0.9% NaCl) and Aβ-1(1µg/µl), Aβ-2(2µg/µl), Aβ-3(3µg/µl), Aβ-4(4µg/µl), Aβ-5(6µg/µl), Aβ-6(8µg/µl) and Aβ-7(10µg/µl) groups obtained by injection of 5 µl per ventricle. Then, AEPs were recorded in freely-moving rats. Latencies and amplitudes of AEPs, evoked power, inter-trial phase synchronization, and auditory evoked gamma responses were obtained in response to auditory stimulus. Furthermore, $A\beta_{1-42}$ levels were determined in the temporal cortex.

Results: $A\beta_{1-42}$ levels were significantly higher in the temporal cortex in Aβ groups compared to the Sham. In frontal and parietal regions, P1N1 amplitudes were significantly decreased in Aβ-3, 4, 5 and 6 groups, and N1P2 amplitudes were significantly decreased in all Aβ groups, whereas in temporal regions, P1N1 and N1P2 amplitudes were decreased in Aβ-2, 3,4,5,6 and 7 compared to the Sham. In the evoked gamma power and phase synchronization of gamma responses, we detected significant decrement after Aβ-4 group, whereas a significant decrement in the filtered gamma responses was observed in Aβ groups compared to the Sham.

Conclusions: AEPs might be used as a biomarker to determine the $A\beta_{1-42}$ related neuronal degeneration in the auditory networks.

Keywords: Alzheimer's disease; amyloid beta peptide; auditory evoked potentials; gamma oscillation; rat

1. Introduction

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder which gradually deteriorates memory and functional ability. The disease is characterized by two exclusive pathologies in the brain: accumulation of numerous amyloid plaques and neurofibrillary tangles, the former starts earlier than the latter [1, 2]. These accumulated proteins, such as amyloid beta (Aβ) peptides, are intrinsically cytotoxic in vitro, and this accumulation stimulates synaptic loss, mitochondrial impairment, and apoptosis in neurons, causing ultimately deterioration of neural networks dynamics [3] and also responsible for early cognitive decline in AD patients [4-6]. Most of the current studies related to AD treatment have been focused at developing drugs that inhibit accumulation, clearance or toxicity of Aβ peptide [7-9]. Unfortunately, there are no effective therapeutic modalities that are clinically beneficial, but if the possible changes in the neural network caused by the accumulation of Aβ peptide can be detected in early stages of the disease, interventions may be developed to provide effective treatment of AD before permanent neurodegeneration occurs [10]. On the other hand, we need to underline that the findings of complementary therapies and some non-pharmacological studies such as brain stimulation techniques have promising outcomes on the treatment of AD related symptoms [11-13]. For clinical and experimental applications, electroencephalography (EEG) is a key technique to analyze the integrity of brain, sensory and/or cognitive systems and functional connectivity between different brain regions, and also the lowest cost and a noninvasive method [14-16]. Therefore, it is of great importance that the diagnosis of AD characterized by the accumulation of $Aβ_{1-42}$ peptide and loss heavy of neuron and synapse in the cerebral cortex is detected by evaluating brain oscillations directly.

Brain oscillations have been reported to play a role in an important part of mechanisms underlying sensory and/or cognitive functions. These oscillations differ as magnitude and

frequency depending on brain arousal state, their location and the exact nature of their neuronal network sources [17]. In the literature, spontaneous EEG and event-related potentials (ERPs) studies have been preferred generally in AD animal models, but there are not enough studies related to evoked potentials (EPs). In one of these studies, it has been reported that theta activity in spontaneous EEG was decreased, whereas beta and gamma activities were significantly increased in AD transgenic mouse models [18]. Pena-Ortega, (2013) also demonstrated that cerebral injection of Aβ peptide reduced theta activity by disturbing neuronal network dynamics, and ultimately caused cognitive dysfunction in animals [19]. In other studies, it was detected that the intracisternal injection of Aβ did not have any effect spontaneous hippocampal theta activity, but caused to disturbed sensory evoked-induced theta activity [20]. In contrast, some studies argue that spontaneous EEG pattern is characterized by an increment at slow frequency components and a decrement at fast frequency components in AD animal models obtained by damaging cholinergic neurons, in addition to the decreased cholinergic activity and cognitive dysfunction [21]. In the light of these studies, it could be said that the $Aβ_{1-42}$ peptide caused to deterioration of neural network dynamics.

To illuminate the mechanism of causation of AD, it is necessary to determine how $Aβ_{1-42}$ peptide affects neural networks. To date, there is no study to evaluate the effects of $Aβ_{1-42}$ peptide injections into rat cerebral ventricles at increasing concentrations on auditory sensory networks have not been studied in awake rats. Considering the temporal lobe and associated cortices are the important brain regions among the main and early targets of $Aβ_{1-42}$ aggregates, and the neuronal oscillations are the phase-locked to the stimulus involved in the basis of EPs, it could be said that auditory evoked potentials (AEPs) might be used the detection of early electrophysiological changes induced by administration of aggregated $Aβ_{1-42}$ peptide. Also, there are a lot of studies which show that hearing loss might be associated with the development and progression of cognitive impairment and dementia or AD, independent of

age [22, 23], but it must be underlined that there is still no exact evidence of whether hearing loss associated with AD is a causal factor in dementia or is associated with temporal lobe pathologies. On the other hand, we consider that it has lower variability between subjects because sensory EPs do not require any cognitive processing and perception rather than ERPs, thus it may be more useful to detect $A\beta_{1-42}$ peptide related changes on auditory sensory networks via sensory EPs. Therefore, we aimed to determine whether there are any dose dependent effects of $A\beta_{1-42}$ peptide on auditory sensory networks and these possible changes might be used to predict for early signs of $A\beta_{1-42}$ peptide burden and localization in the brain.

2. Materials and Methods

2.1 Animals

The rats were obtained from Akdeniz University Animal Care Unit. Male albino Wistar rats aged 3 months, weighing 250 to 300 g were housed in stainless steel cages and given food and water *ad libitium*. Animals were maintained at 12-h light-dark cycles and a constant temperature of $23\pm1°C$ at all times. The study design was representative illustrated in Fig. 1.

2.2 Ethical Approval

Ethical approval for this work was obtained from Akdeniz University Local Committee on Animal Research Ethics (Ethics Approval Date and Number: 09.11.2015/2015.01.11-3). All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University.

2.3 Animal Preparation

All rats had five days handling. Following handling sessions, the animals were randomly divided into eight groups (n=10 per group); 7 AD groups obtained by intracerebroventricular (i.c.v.) injection of $A\beta_{1-42}$ peptide (Aβ-1, Aβ-2, Aβ-3, Aβ-4, Aβ-5, Aβ-6 and Aβ-7) in different concentrations (1 μg/μl, 2 μg/μl, 3 μg/μl, 4 μg/μl, 6 μg/μl, 8 μg/μl, and 10 μg/μl, respectively) and a sham group (0.9% NaCl). The rat model of AD was established as described previously [24, 25]. $A\beta_{1-42}$ peptide (Sigma, USA) was injected into the lateral ventricles of the rats (5 μl per ventricle for each group). Stock solutions with different concentrations (1 μg/μl, 2 μg/μl, 3 μg/μl, 4 μg/μl, 6 μg/μl, 8 μg/μl, and 10 μg/μl) were prepared in sterile normal saline. To obtain the aggregated form of $A\beta_{1-42}$, the stock solutions were then incubated at 37°C for three days before use.

2.4 Surgery protocols

The details of surgical protocols have been described previously [26]. In briefly, rats were anesthetized with a mixture of ketamine-based anesthetics (ketamine [50 mg/kg] and xylazine [10 mg/kg], intraperitoneally (i.p.)) and fixed on a stereotaxic instrument. Stainless steel screw electrodes were implanted bilaterally over the frontal (AP: 4.5 mm, ML: +2 and -2 mm), parietal (AP: -4.5 mm, ML: +3.5 and -3.5 mm), temporal (AP: -8.0 mm, ML: +6.6 and -6.6 mm) and occipital (AP: -8.0 mm, ML: +4.1 and -4.1 mm) regions, and the reference electrode was inserted into the cerebellum (AP: -12.72 mm, ML: 2.5 mm). Following chronically electrode implantation, either Aβ $_{1-42}$ peptide or saline (0.9% NaCl) was injected into the lateral ventricles (injected coordinates: AP: −0.8 mm, ML: −1.4 mm and +1.4 mm, DV: −4.0 mm) at 1 µl/minute using a Hamilton microsyringe. After administration of solutions and electrode implantation, 1 week was allowed for recovery. After surgery, an otoscopic examination was performed to evaluate tympanic membrane damage before AEPs recordings. No tympanic damage was found in the rats. During recovery, the rats were housed in individual cages with free access to food and water.

2.5 Electrophysiological recordings

The details of electrophysiological recordings have been described previously [26]. In briefly, for the recordings from free-moving rats, chronically implanted electrodes were used. All electrode impedances were less than 10 kOhm. The EEG signal was amplified (Brainamp EEG/EP Amplifier, Brain Products, Munich, Germany), band-pass filtered (0.1-300 Hz) and digitized at a 1000 Hz sampling rate (Brainvision Recorder, Brain Products, Munich, Germany). AEPs were recorded using tones of 8000 Hz at the 85 dB. The repetition rate of auditory stimulus was 1 Hz. The duration of the 85-dB tones was 50 ms and the tones were presented through a loudspeaker at a distance of approximately 15 cm from the ear of the rat. The EEG data were processed in 1000 ms epochs (500 ms pre-stimulus/500 ms post-stimulus). The averaging of 100 responses were performed with a BrainVision Analyzer

(Brain Products GmBH). Peak latencies of the components were measured from the stimulus artifact to the peak in milliseconds. Amplitudes were measured as the voltage between successive peaks. Measurements were made on one negative (N1) and two positive (P1, P2) potentials which are seen in all groups.

2.6 Data analysis

2.6.1 Event-related spectral perturbations (ERSP) analysis

Time-frequency analysis was applied for all epochs between 4 to 48 Hz using Morlet-based wavelets transform with 3 cycles and within 2 ms- sliding windows between -500 to 500 ms (EEGLAB) [27]. Spectral analysis was computed on the wavelet-transformed epochs for each stimulus at each time point and wavelet frequency to yield time-frequency maps. The color at each image pixel indicates amplification (in dB) at a given frequency and latency to the time locking stimulus. Spectral analysis was used to determine the dominant frequencies in the AEPs during the experiment. The peak powers at 28-48 Hz individual frequency were extracted for statistical assessment. Therefore, we used each rat's peak power at the gamma frequency band.

2.6.2 Inter-trial coherence (ITC) analysis

Inter-trial coherence (ITC) indicates that the EEG activity at a given time and frequency in single trials becomes phase-locked. The ITC measure takes values between 0 and 1. A value of 0 represents absence of synchronization between EEG data and the time locking stimulus; a value near 1 indicates phase synchronization. Here, we calculated the ITC using (EEGLAB) [27] as follows: For j=1 to N trials,

$$ITC\ (t,f) = \left\| \frac{1}{N} \sum_{i=1}^{N} e^{j\varphi j(t,f)} \right\|$$

where $\varphi j(t,f)$ is the phase of the wavelet at time t and frequency f. All ITC values were baseline corrected over -300 ms to -50 ms and were computed each rat for grand average. We used each rat's peak-frequency at 28-48 Hz frequency band.

2.6.3 Digitally filtered auditory evoked gamma oscillatory responses

Digital filtering of AEPs was performed with BrainVision Analyzer software (Brain Products GmBH). Each rat's averaged evoked responses were digitally filtered in the 28-48 Hz frequency range. The maximum peak-to-peak amplitudes for each rat's averaged gamma (28-48 Hz) responses were analyzed. The largest peak-to-peak value in these frequency ranges in terms of microvolt found in the time window between 0 and 200 ms.

2.7 Measurement of $A\beta_{1-42}$ peptide levels

After the electrophysiologic recordings, the rats were deeply anesthetized (20% urethane, 5 mL/kg, i.p.) and a cardiac cannula was placed. Brain tissues were perfused with heparinized isotonic (heparin (Innohep, Leo Pharma, Denmark), anticoagulant, and isotonic solution (0.9% NaCl) were administered via the cannula. Temporal cortex was immediately dissected for enzyme-linked immunosorbent assay (ELISA) analysis. $A\beta_{1-42}$ levels in the supernatants of tissue lysates were measured using a commercially available ELISA kit (SensoLyte Anti-Mouse/Rat b-amyloid (1-42) Quantitative ELISA *Colorimetric*, AnaSpec, Fremnot, CA, USA) in accordance with the manufacturer's instructions.

2.8 Statistical analysis

All statistical analyses were performed using SPSS 18.0 (SPSS, Chicago, IL, USA) software for Windows. $A\beta_{1-42}$ levels were analyzed using analysis of variance (ANOVA). Post-hoc comparisons were analyzed using the Bonferroni test. A repeated measure of ANOVA was used to determine the statistical significance differences of latencies, peak-to-peak amplitudes, spectrograms, ITC and filtered auditory evoked gamma power response to auditory stimulus over different locations and between the groups. During the analysis,

repeated measures of ANOVA included the between-subjects factor as group (Sham, Aβ-1, Aβ-2, Aβ-3, Aβ-4, Aβ-5, Aβ-6 and Aβ-7), and within-subject factors as 8 electrode sites (FrA1, FrA2, PtA1, PtA2, TeA1, TeA2, V1 and V2). Greenhouse–Geisser-corrected p values were reported. Results are expressed as mean ± standard deviation (SD). Significance levels were set at $p < 0.05$.

3. Results

The animals were apparently in good condition during experiment. No significant difference could be observed in the body weight change among different experimental groups (data not shown).

3.1 Results of $A\beta_{1-42}$ peptide levels

ANOVA for $A\beta_{1-42}$ peptide levels revealed a significant treatment effect [F(7.49) = 33,61, p<0.001]. Values of $A\beta_{1-42}$ peptide levels in the temporal cortex (mean ± SD) in the sham and $A\beta_{1-42}$ treated groups are given in Fig. 2. Post-hoc comparison tests indicated that $A\beta_{1-42}$ peptide levels were significantly increased in all $A\beta_{1-42}$-injected groups compared to the sham group, and also there were significant increment in Aβ-4, 5, 6 and 7 groups compared with Aβ-1group.

3.2 Results of latency and amplitude values of AEPs

P1, N1 and P2 components of AEPs for all experimental groups are presented in Fig. 3. Measurement was made on one negative and two positive potentials, which were seen in all of the groups. The means and SD of peak latencies of AEPs components of the all groups are shown in Tab. 1. There were found to be a significant effect of the electrode localization on all latencies values as a result of repeated ANOVA analysis [F (7,392)=12.71; F (7,392)=23.94; F (7,392)=5.79; p<0.001]. Post-hoc comparisons of the electrode regions were found to have shorter latency values in the temporal and occipital regions than in the other regions (p<0,05). There were no significant changes in P1, N1 and P2 latency values between the experimental groups [F (7,56)=1.47; F (7,56)=0.96; p>0.05].

The means and SD of peak-to-peak amplitudes of AEPs components of the all groups are shown in Tab. 2. In the result of repeated ANOVA, it was determined significant difference between electrode regions for maximum peak-to-peak P1N1 and N1P2 amplitudes [F (7,392)=51.31; F (7,392)=31.53; p<0.001]. Post-hoc comparisons revealed that the P1N1

response was in the lowest in the occipital regions and the N1P2 response was in the highest temporal regions. Furthermore, there was also observed a significant interaction effect for location × group in maximum peak-to-peak P1N1 and N1P2 amplitudes [$F_{(49,56)} = 3.56$ $p<0.001$; $F_{(49,56)} = 1.59$; $p<0.01$], thus showing that the observed differences between groups were not found in all regions. For more detailed information about the differences in the both interaction effects and between the groups, the changes in P1N1 and N1P2 amplitudes were analyzed separately for different electrode regions. In a more detailed analysis, it were observed that a statistically significant decrement in the P1N1 amplitude at the frontal and parietal regions in the Aβ-3, 4, 5, and 6 groups, and at the temporal regions in the Aβ-2, 3, 4, 5, 6, and 7 groups compared with Sham group. A significant decrement were detected in N1P2 amplitude at the frontal and parietal regions in all Aβ groups, and at the temporal regions in Aβ-2, 3, 4, 5, 6 and 7 groups compared with Sham group. No significant differences were detected both P1N1 and N1P2 amplitudes at the occipital regions in the all groups.

3.3 Results of event-related spectral perturbations (ERSP)

Fig. 4 illustrates the grand average of time–frequency planes showing the post-stimulus enhancement of gamma responses in auditory stimulus for sham and all Aβ groups at TeA1 location. The means and standard deviations of maximum gamma band spectrograms values of AEPs in all groups are shown in Tab. 3. The grand average of the ERSP values indicates that the auditory stimulus results in a strong gamma-response power within 100 ms following stimulation in both sham and $Aβ_{1-42}$ treated groups, but stronger in the sham group. In the result of repeated ANOVA, there was no significant location x group interaction, and therefore differences between groups were examined independently of electrode location. Post-hoc comparisons indicated that gamma response power is significantly reduced in the

Aβ-4, 5, 6 and 7 groups compared to the sham group (p<0.001) and in the Aβ7 group compared to the Aβ1 group (p<0.001).

3.4 Results of event-related inter-trial coherence (ITC)

Fig. 5 illustrates the grand average of time–frequency planes showing the ITC of gamma responses in auditory stimulus for sham and all Aβ groups at TeA1 location. The means and standard deviations of maximum gamma band ITC values of AEPs in all groups are shown in Tab. 4. Results indicates that, in the 0-100 ms time window, auditory stimulus leads to a strong gamma-phase lock in both sham and $Aβ_{1-42}$ treated groups, but stronger in the sham group. In the result of statistical analysis, it was observed significant differences between groups [$F(7,49)= 9.49$ $p<0.001$], but there was no significant differences at location x group interaction. Therefore, the observed differences between groups were examined independently of electrode location. Post-hoc comparisons indicated that gamma phase locking values were significantly reduced in the Aβ-4, 5, 6 groups compared to the sham and Aβ1 groups and in the Aβ-7 compared to the sham and Aβ-1, 2, 3 groups.

3.5 Results of digitally filtered auditory evoked gamma oscillatory responses

Fig. 6 illustrates the grand average of filtered (28-48 Hz) gamma responses of sham and all $Aβ_{1-42}$ treated groups at TeA1 location. The means and standard deviations of filtered (28-48 Hz) auditory evoked gamma oscillatory responses values in all groups are shown in Tab. 5. Repeated ANOVA indicated that there were significant alteration between groups [$F(7,56)= 13.52$ $p<0.001$], electrode location [$F(7,392)= 45.91$ $p<0.001$], and location x group interaction [$F(49,56)= 5.78$ $p<0.001$]. Post-hoc comparisons revealed that evoked gamma responses were decreased in all $Aβ_{1-42}$ treated groups compared to sham in frontal, parietal and occipital, but there was no significant changes among $Aβ_{1-42}$ treated groups. In temporal region, evoked gamma responses were decreased in all $Aβ_{1-42}$ treated groups compared to

sham, and also there were significant reductions in Aβ-7 group compared to other $A\beta_{1-42}$ treated groups.

4. Discussion

In our previous study, we reported that spontaneous delta activity is altered only after a significant accumulation of $A\beta_{1-42}$, whereas all concentrations of $A\beta_{1-42}$ peptide (between 1 and 10 μg/μl x 5 μl per each ventricle) have effects characterized by a reduction in the spontaneous gamma activity. Also, we observed that i.c.v. application of $A\beta_{1-42}$ peptide has led to AD-like memory deficits in the rats [26]. In this study, AEPs were recorded from the freely moving rats to examine the effect of $A\beta_{1-42}$ peptide on the sensory system and to determine the possible associated biomarkers depending on the accumulation of $A\beta_{1-42}$ peptide. For this purpose, we used the same AD rat model which is based on i.c.v. injection of aggregated $A\beta_{1-42}$ peptide into the animal brain as in our previous study.

To determine $A\beta_{1-42}$ peptide induced alterations in AEPs, peak latency and peak-to-peak amplitude values of responses to auditory stimuli were calculated for each group. Examining the responses to auditory stimuli, it was observed that there was no statistically significant difference between groups in peak latency of AEPs. These findings clearly indicate that the rate of auditory information processing is not affected by the application of $A\beta$ peptide. In consistent with our results, it has been indicated that latencies of auditory sensory components did not change in EPs recorded from AD patients [28]. On the other hand, examining the peak-to-peak amplitude of the responses to auditory stimuli, it was found that there were statistically significant differences in the amplitude of AEPs. As previously reported, the P1N1 amplitude is associated with physical properties such as early sensory functions and the intensity, frequency, spectral and temporal characteristics of the stimulus, and the N1P2 amplitude is associated with later sensory functions such as processing and interpretation of auditory information [29]. Thus, our results indicate that the administration of $A\beta_{1-42}$ peptide leads to deterioration of the sensory system integrity. The significant amplitude changes in AEPs also point out that the administration of $A\beta_{1-42}$ peptide may

reduce the amount of neuronal populations actively engaged in these processes or deteriorate synchronously firing properties of neurons and causes neurodegeneration in stimulus related regions. Since the temporal cortex and associated regions are more sensitive to the neurodegeneration than other brain regions in the early stages of AD, auditory evoked responses are expected to be more affected in the temporal cortex and associated regions.. Consequently, the sensitivity emerging in the temporal regions is thought to be related to the modality of the stimulus which is used rather than the Aβ burden, besides it would potentially be a useful biomarker to detect early degeneration related to $A\beta_{1-42}$ peptide.

On the other hand, as known, the EEG consists of the summed electrical activities of generator neuronal clusters with rhythmic activity in many frequency bands. These brain oscillations are called spontaneous brain activity resulting from a completely random activity. However, a sensory stimulus can induce synchronization in the activity of these neuronal clusters. This synchronization provides occurrence of evoked responses which have a certain pattern and time in the brain. Hence, it could be concluded that these evoked responses, representing the temporal activity of neuronal clusters, are originated from the transitions from the irregular activity of neuronal clusters to regular activity related to stimulus [30, 31]. One of these oscillations is the gamma band (30-70 Hz) oscillation. Gamma oscillation is a multifunctional brain activity that can be selectively observed in different subcortical and cortical regions; as opposed to representing a specific function of the central nervous system rather than the other frequency bands. Specifically, it is thought to be involved in information processing in cortical networks [32]. In the literature, is has been shown that the gamma oscillation synchronization is an important and fundamental process for brain function, which emerges within the first 150 ms following stimulation [33, 34]. In human studies, researchers also reported that synchronization of neural activity was disturbed in AD patients, which observed reduced functional communication within and between cortical regions [35]. In

particular, the early stages of AD have been characterized by a decrement in functional connectivity between specific brain regions related to cognition [36].

We detected i.c.v. application of $A\beta_{1-42}$ peptide has caused significant changes in the auditory evoked responses. Especially, the most obvious changes between groups were observed in the gamma band (28-48 Hz), which emerges within the first 100 ms following stimulation. $A\beta$ groups have a trend to display lower auditory evoked gamma response than the sham group. Despite the fact that no regional differences in the gamma activity depending on the accumulation of $A\beta_{1-42}$ peptide have been noticed, it has caused similar changes in ERSP and ITC values, and progressive decrement was observed in auditory evoked gamma responses in power and in phase synchrony upon presentation of stimulation in $A\beta$ groups than the sham group. Also, this decrement did not reach a significant level in the group comparisons up to 4 μg/μl concentration of $A\beta_{1-42}$ peptide x 5 μl. On the other hand, it has been detected that there is a statistically significant relationship between alteration of gamma oscillation and accumulation of $A\beta$ peptide. In a study, it was observed that the accumulation of $A\beta$ peptide was decreased by modulation of gamma oscillations. The possible cause of this decrement was reported to be suppressed amyloidogenesis and improved amyloid endocytosis due to microglial activation [37]. Additionally, gamma oscillations are known to occur by the synchronous activity of excitatory and inhibitory (E/I) synaptic interactions in the brain [38, 39]. These E/I synaptic interactions have been reported to be affected by the accumulation of hyperphosphorylated tau peptides and $A\beta$ peptide, the most important neuropathological markers of AD [40, 41]. In addition, it was reported that transcranial magnetic stimulation (TMS) techniques have an important role in the detailed assessment of the neurophysiological mechanisms underlying AD. As a result of these studies, it is hypothesized that AD is thought to be a complex neurodegenerative disease involving different neurotransmitter systems [42]. On the other hand, the findings of some TMS studies indicated that the resting motor

threshold is generally reduced in AD, which is interpreted as a marker of increased motor cortex excitability [43-45]. These results also support that an imbalance of E/I synaptic interactions in the cerebral cortex is observed in AD pathology. Considering all these findings, it is conceivable to conclude that the degeneration in these E/I synaptic interactions due to the accumulation of Aβ peptide is likely to be the underlying cause of the reduction in the evoked gamma responses observed in this study. Moreover, it is likely to put forward that the $A\beta_{1-42}$ peptide disrupts the synchronous activity between the neural clusters and eliminates the regularity associated with the sensory stimulation with the decreases observed in the ERSP and ITC values in the Aβ-treated groups with high doses.

4.1 Limitations of the study

The complexity of AD pathology is the main limitation of this study. The results of our study have been obtained from the rats which have only Aβ pathology, so the changes in AEPs are associated with the Aβ pathology. However, considering the complex pathology and age-related hearing problems in AD patients, it seems not possible to say how the investigated parameters will change in AD patients. Thus, further studies, specifically human studies, are needed to obtain direct evidence about AD-related changes in the auditory sensory networks, and for clinical applications.

5. Conclusions

The changes induced by $A\beta_{1-42}$ peptide in the amplitude of AEPs indicate that the accumulation of $A\beta_{1-42}$ peptide has an important effect on auditory sensory system functions, and cause disruption in the integrity of sensory system. Moreover, the decrement of amplitude rather than latency empowers the idea that accumulation of $A\beta_{1-42}$ peptide leads to a decrement in the amount of neuronal population taking active roles in sensory processes, and thus neurodegeneration in the related regions rather than the rate of information transmission and processing. In addition, the findings of evoked gamma responses indicate that the $A\beta_{1-42}$

peptide leading to a global effect in the brain can be examined by the evoked gamma power and phase synchrony, while $A\beta_{1-42}$ peptide location might be determined by evaluating the filtered gamma band responses due to observed regional differences in this activity.

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Figure Legends

Fig. 1. A schematic flow chart of the experimental design.

Fig. 2. Aβ1-42 peptide levels in the temporal cortex obtained using ELISA in all groups (n=8 per group). Aβ1-42 peptide levels were significantly increased in all Aβ1-42-injected groups compared to the sham group (***: $p<0.001$). Mean ± SD values are given on the bar graph for each group.

Fig. 3. Grande average of AEPs waveforms evoked by auditory stimulus at the TeA1 electrode location. Different colors represent different groups. Time 0 is the stimulus onset and N1 is the first negative wave.

Fig. 4. Grand average of ERSP time-frequency matrices calculated by wavelet-based analysis of the averaged evoked potentials showing relative changes in total power compared with the baseline period at all electrode locations. Power (in dB) is indicated by color code with warmer colors representing higher power values (color bar at the right side of each panel). It was found that a certain amount of $A\beta_{1-42}$ peptide leads to a decrease in gamma-band power compared to the Sham group.

Fig. 5. Grand average of time-frequency plots showing phase-locking (ITC) values for auditory stimulus at all electrode locations. Phase-locking is indicated by color code with warmer colors representing higher phase stability across trials (color bar at the right side of each panel). ITC was detected to be affected by injection of $A\beta_{1-42}$.

Fig. 6. Grand average of filtered (28-48 Hz) auditory evoked gamma oscillatory responses of all groups at the TeA1 electrode location. Evoked gamma responses were significantly decreased in all $A\beta_{1-42}$ treated groups compared to Sham group.

Authors' Responses to Reviewers

We thank the reviewers for critical reading of the paper and helpful comments. We performed the required corrections and improved the relevant sections to address their questions and comments point-by-point. So, we hope that the responses have the potential to match these questions satisfactorily.

Sincerely

Corresponding Author

<u>**Reviewer #1:**</u>

1. Authors have revised manusript according to reviewers' comments. The paper can be accepted for further processing in the revised version.

Response: Thank you for your contributions and thoughts.

<u>**Reviewer #3:**</u>

1. The article has been corrected as recommended. The work is interesting and presents new information. The presented work has great potential in terms of further research development. Currently, the work in the form presented can be accepted for printing.

Response: Thank you for your contributions and thoughts.

<u>**Reviewer #5:**</u> The authors have adequately addressed my concerns, thus improving the quality of this manuscript. I do not have further requests, except for a minor remark. Regarding the transcranial magnetic stimulation studies in AD, the authors report three old studies only (Alagona, et al, 2004; Ferreri, et al. 2003; Pennisi, et al. 2002). I suggest to replace them with a more recent and comprehensive review (i.e. Pennisi G, et al. J Neural Transm 2011), which includes also the three aforementioned studies.

Response: Thank you for your contributions and thoughts. And also, we performed necessary corrections in line with your comment. We added a sentence as seen below.

"In addition, it was reported that transcranial magnetic stimulation (TMS) techniques have an important role in the detailed assessment of the neurophysiological mechanisms underlying AD. As a result of these studies, it is hypothesized that AD is thought to be a complex neurodegenerative disease involving different neurotransmitter systems [42]".

[42] Pennisi G, Ferri R, Lanza G, Cantone M, Pennisi M, Puglisi V, et al. Transcranial magnetic stimulation in Alzheimer's disease: a neurophysiological marker of cortical hyperexcitability. J Neural Transm (Vienna). 2011;118:587-98.

Conflict of interest

The authors declare no conflicts of interest.

Financial disclosure

Fig. 1

Handling Session   Recovery Period   Data Analysis
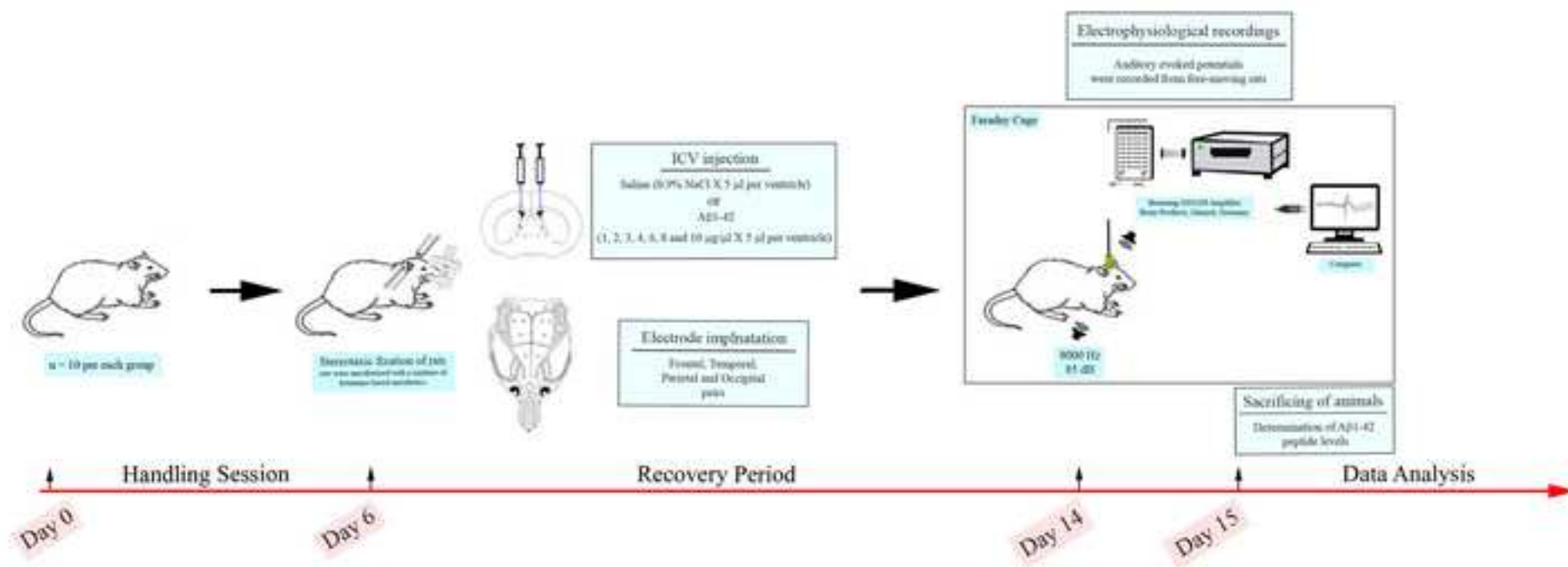
Day 0   Day 6   Day 14   Day 15

Fig. 2
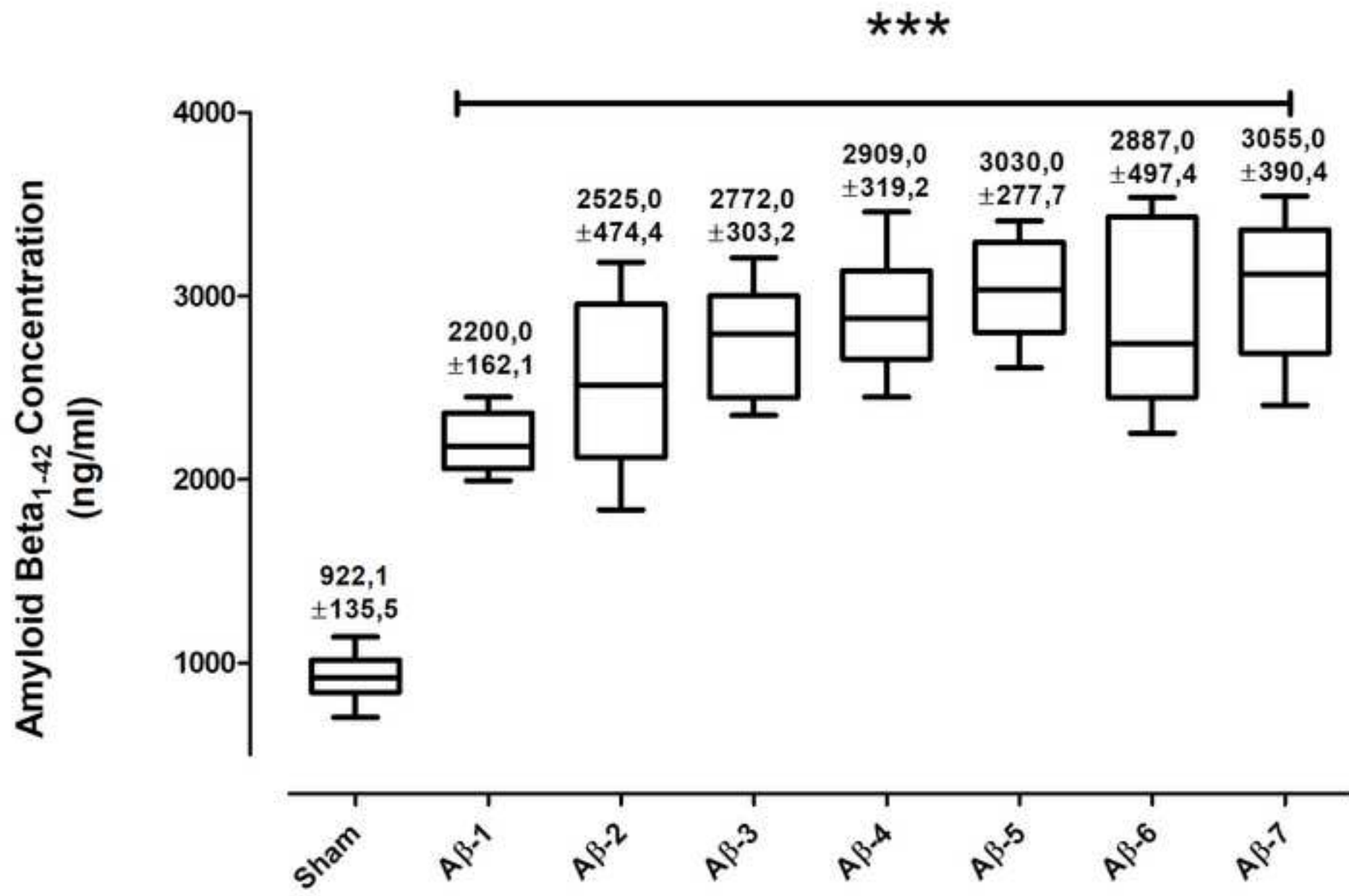**Click here to download high resolution image**
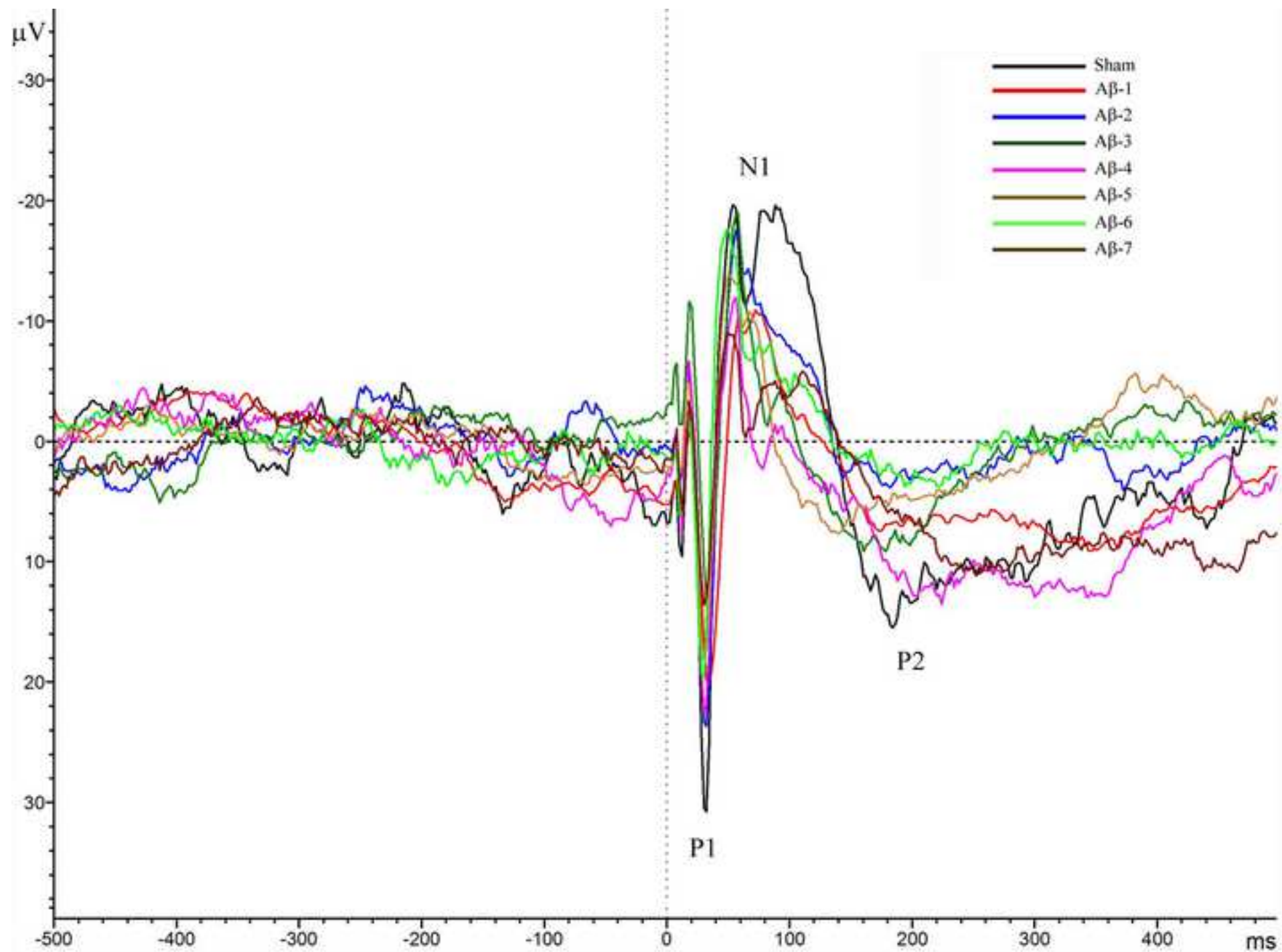
Fig. 2

Fig. 3
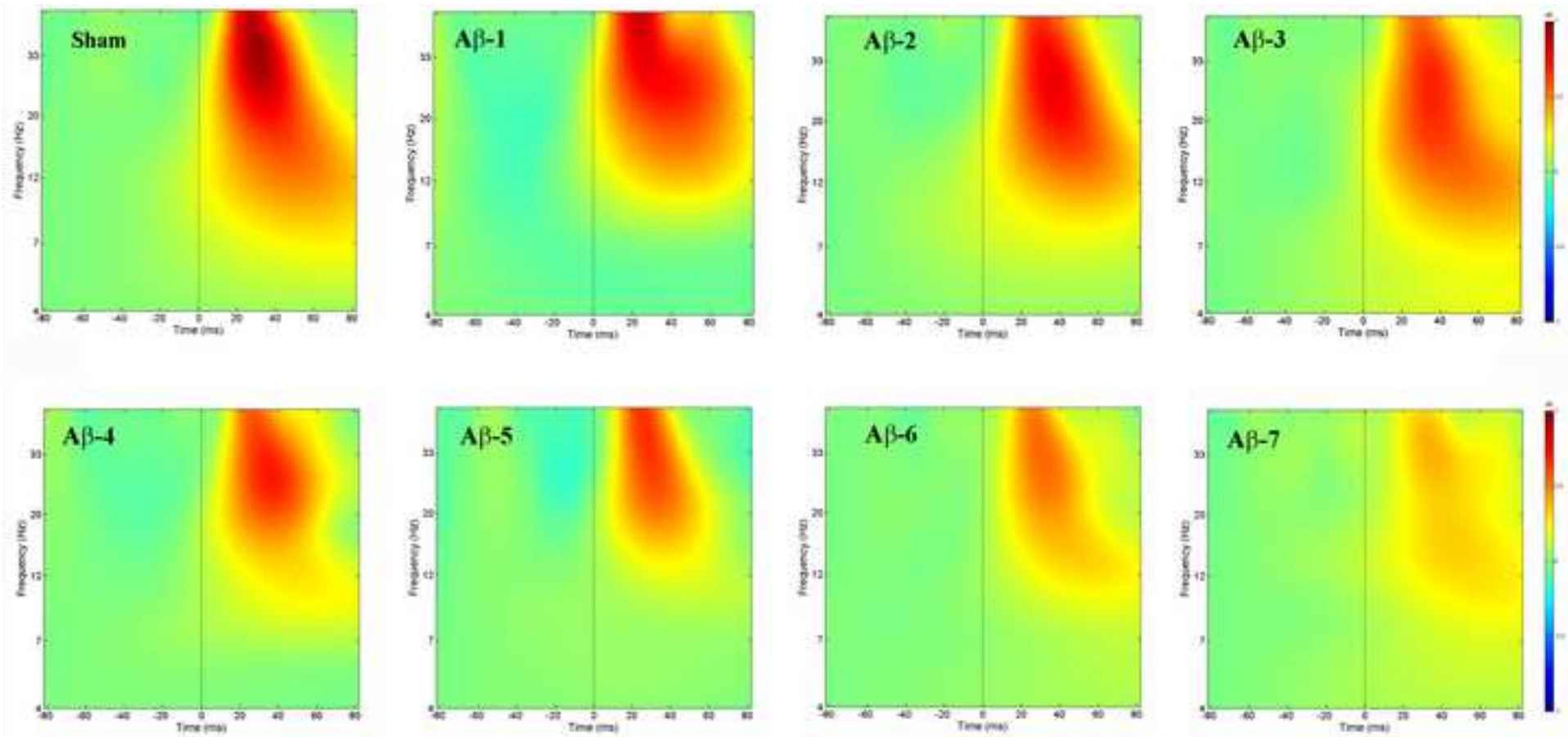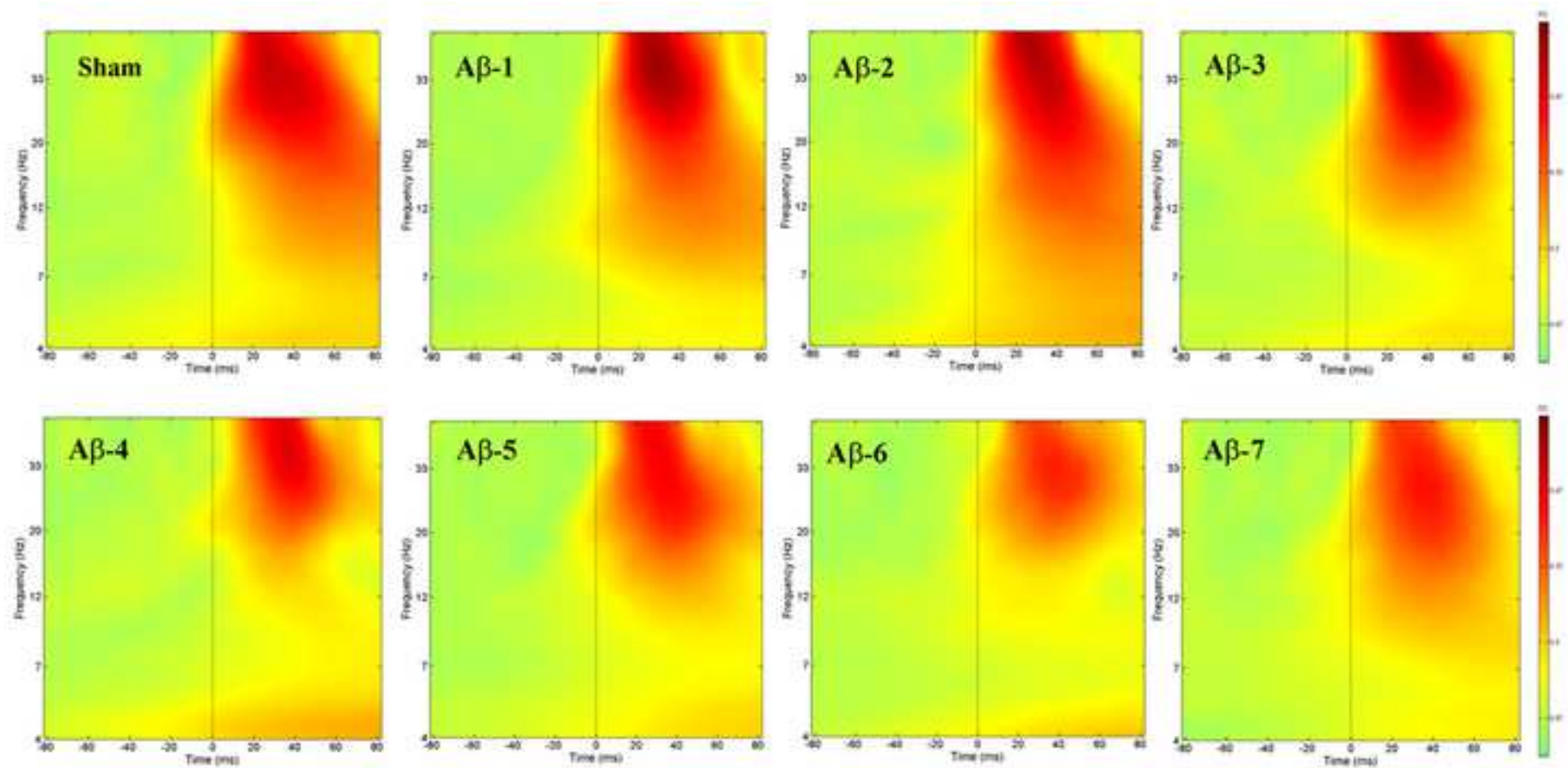**Click here to download high resolution image**

Fig. 4
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Tab. 1

Tab. 1. The means and standard deviations of peak latencies of AEP components in Sham and Aβ groups. There was no main group effect in terms of peak latencies of AEPs components between all groups.

	Latency (ms)											
	FrA1(ms)			**FrA2(ms)**			**PtA1(ms)**			**PtA2(ms)**		
Groups	**P1**	**N1**	**P2**	**P1**	**N1**	**P2**	**P1**	**N1**	**P2**	**P1**	**N1**	**P2**
Sham	31,00±5,55	82,50 ±18,72	199,25 ±36,19	32,00 ±5,95	87,85 ±23,16	195,00 ±32,12	31,00 ±4,90	83,25 ±18,64	192,75 ±22,06	31,75 ±7,05	89,00 ±19,91	188,25 ±29,73
Aβ-1	38,75 ±4,13	81,00 ±8,75	187,75 ±22,18	39,00 ±4,14	84,88 ±13,54	193,63 ±17,97	40,00 ±5,13	85,25 ±8,21	192,50 ±22,03	38,25 ±3,92	88,00 ±16,49	201,25 ±20,37
Aβ-2	33,00 ±3,66	90,25 ±9,94	186,00 ±21,17	33,25 ±2,60	92,50 ±10,84	192,25 ±17,48	33,25 ±2,60	82,25 ±11,97	188,00 ±17,17	33,75 ±2,71	74,50 ±11,10	184,25 ±13,71
Aβ-3	35,50 ±3,66	69,50 ±11,55	175,25 ±18,48	35,75 ±3,92	69,75 ±12,35	176,00 ±18,67	36,25 ±3,92	69,00 ±13,82	180,25 ±20,99	36,25 ±4,46	70,50 ±12,77	185,00 ±12,96
Aβ-4	33,50 ±6,39	75,63 ±17,74	178,25 ±12,98	36,00 ±8,75	75,13 ±14,79	183,50 ±9,30	35,00 ±5,45	76,50 ±14,61	191,50 ±11,05	37,75 ±4,20	81,25 ±16,03	194,75 ±8,48
Aβ-5	38,50 ±4,50	89,75 ±17,38	179,50 ±13,13	39,00 ±5,13	93,75 ±15,29	192,50 ±12,95	38,25 ±4,71	86,75 ±9,97	185,25 ±20,99	40,00 ±5,45	92,75 ±17,30	195,25 ±18,23
Aβ-6	34,50 ±5,73	82,75 ±14,14	181,75 ±10,66	37,00 ±5,01	88,00 ±11,51	186,13 ±9,42	34,00 ±4,00	81,50 ±21,75	184,75 ±11,90	33,50 ±3,34	80,50 ±17,46	186,50 ±14,01
Aβ-7	36,50 ±5,10	95,00 ±7,25	187,25 ±16,07	37,25 ±6,76	98,75 ±6,14	189,25 ±8,28	36,00 ±3,55	87,50 ±11,94	192,25 ±12,49	36,25 ±3,28	93,00 ±10,85	187,25 ±13,52
	TeA1(ms)			**TeA2(ms)**			**V1(ms)**			**V2(ms)**		
	P1	**N1**	**P2**	**P1**	**N1**	**P2**	**P1**	**N1**	**P2**	**P1**	**N1**	**P2**
Sham	31,50 ±2,78	75,50 ±18,26	199,75 ±28,55	31,50 ±2,78	75,25 ±24,96	192,75 ±24,52	31,25 ±3,20	77,50 ±21,80	186,00 ±22,32	34,00 ±7,78	79,50 ±28,52	196,50 ±26,55
Aβ-1	34,25 ±2,71	78,75 ±18,14	188,00 ±22,42	36,50 ±3,16	80,00 ±25,63	192,50 ±27,33	38,50 ±6,48	98,50 ±27,91	184,00 ±14,06	37,25 ±3,99	81,88 ±19,63	184,25 ±18,99
Aβ-2	32,25 ±2,49	72,13 ±8,39	182,25 ±17,38	32,50 ±2,56	78,75 ±10,47	189,75 ±11,83	31,75 ±2,25	71,50 ±11,80	173,88 ±8,56	32,00 ±1,85	75,00 ±9,32	181,13 ±6,62
Aβ-3	35,75 ±3,62	65,75 ±12,02	181,50 ±15,67	35,50 ±4,87	64,25 ±8,78	186,50 ±17,26	36,50 ±4,50	59,25 ±3,85	178,00 ±15,82	36,00 ±5,24	58,50 ±7,98	187,25 ±15,00
Aβ-4	29,75 ±2,71	65,00 ±14,74	183,25 ±16,35	29,50 ±2,56	62,38 ±12,42	183,75 ±16,88	35,88 ±4,85	72,63 ±13,80	177,00 ±13,05	32,75 ±5,44	66,00 ±18,45	184,75 ±14,14
Aβ-5	31,75 ±3,28	66,50 ±10,13	173,25 ±14,73	31,75 ±2,92	64,75 ±8,00	182,25 ±9,41	36,00 ±5,95	67,75 ±13,16	174,00 ±12,56	35,00 ±5,66	63,25 ±14,73	182,25 ±11,29
Aβ-6	29,75 ±1,28	65,25 ±18,94	175,00 ±11,06	30,75 ±1,83	61,75 ±14,68	179,75 ±12,58	31,50 ±2,07	67,75 ±13,41	178,00 ±13,40	31,25 ±1,49	60,38 ±15,84	178,50 ±8,86
Aβ-7	34,00 ±7,01	80,25 ±17,84	182,00 ±16,66	35,50 ±4,63	74,50 ±20,61	185,00 ±11,71	33,25 ±3,69	66,75 ±22,01	176,25 ±12,40	34,50 ±4,75	73,25 ±21,93	180,25 ±12,12

Tab. 2

Tab. 2. The means and standard deviations of peak-to-peak amplitudes of AEP components in Sham and Aβ groups. Bold indicates a significant difference in Aβ groups compared with the Sham group (Greenhouse-Geisser corrected, p < 0.001)

Peak-to-peak Amplitudes (µV)							
FrA1(µV)		FrA2(µV)		PtA1(µV)		PtA2(µV)	
P1N1	**N1P2**	**P1N1**	**N1P2**	**P1N1**	**N1P2**	**P1N1**	**N1P2**
Groups							
Sham 55,26±20,32	34,52±7,82	55,11±26,12	33,16±13,34	54,94±24,01	27,27±8,93	58,94±31,89	32,73±19,05
Aβ-1 39,30±16,37	**17,12±9,31**	39,41±16,76	**16,68±7,56**	39,56±15,29	**16,24±8,81**	45,10±23,90	**18,45±11,59**
Aβ-2 32,56±8,71	**12,48±5,16**	30,14±7,25	**10,49±5,53**	36,27±13,32	**15,60±8,69**	32,10±11,74	**13,62±5,06**
Aβ-3 **26,96±7,61**	**11,60±6,66**	**25,98±7,31**	**12,38±6,17**	**29,18±5,82**	**18,64±8,87**	**32,91±5,21**	**15,65±7,92**
Aβ-4 **28,39±9,92**	**15,06±7,77**	**34,75±8,93**	**14,06±6,87**	**30,47±11,69**	**16,38±12,16**	**28,05±8,85**	**15,26±7,06**
Aβ-5 **29,69±7,35**	**10,20±5,12**	**23,80±5,64**	**6,12±3,48**	**32,83±8,19**	**11,34±7,64**	**29,16±10,15**	**10,77±4,02**
Aβ-6 **29,90±15,58**	**17,91±9,17**	**29,68±16,97**	**13,57±6,18**	**25,84±9,72**	**14,47±7,69**	**28,73±10,74**	**13,16±9,26**
Aβ-7 49,87±18,17	**24,66±12,20**	36,93±16,93	**16,68±8,71**	42,48±14,36	**19,58±7,85**	35,56±20,75	**12,80±8,97**
TeA1(µV)		TeA2(µV)		V1(µV)		V2(µV)	
P1N1	**N1P2**	**P1N1**	**N1P2**	**P1N1**	**N1P2**	**P1N1**	**N1P2**
Groups							
Sham 64,83±30,53	48,96±26,76	68,28±36,52	51,76±35,62	30,30±19,50	22,69±12,66	33,79±20,79	21,54±15,35
Aβ-1 40,18±19,16	28,11±12,66	40,79±19,12	29,45±11,62	17,02±11,47	6,55±3,87	15,99±4,56	11,89±8,17
Aβ-2 **38,92±12,16**	**20,13±8,62**	**39,30±9,76**	**20,77±8,08**	19,18±9,17	12,82±7,47	17,00±5,14	7,32±2,89
Aβ-3 **30,72±8,07**	**24,74±10,26**	**31,10±8,24**	**23,14±7,82**	19,57±4,64	10,29±4,09	21,25±9,22	10,86±8,54
Aβ-4 **33,63±14,81**	**25,12±18,80**	**37,32±10,88**	**23,60±13,45**	18,49±4,84	9,97±4,01	20,41±7,34	9,56±4,29
Aβ-5 **29,79±12,04**	**17,58±10,16**	**31,87±8,67**	**20,38±8,99**	16,87±6,22	7,16±5,58	14,07±5,69	8,40±3,89
Aβ-6 **38,28±12,36**	**21,88±16,16**	**35,83±14,72**	**20,81±16,72**	21,29±6,21	10,90±6,00	28,89±11,49	17,38±11,97
Aβ-7 **24,16±13,48**	**19,93±9,45**	**23,12±14,42**	**19,88±11,43**	15,27±7,85	9,90±4,08	15,58±13,92	8,64±8,04

Tab. 3

Tab. 3. Means and standard deviations of maximum gamma band spectrograms values of AEPs in Sham and Aβ groups. Bold indicates a significant difference compared to the Sham (Greenhouse-Geisser corrected, p < 0.001).

Groups	FrA1	FrA2	PtA1	PtA2	TeA1	TeA2	V1	V2
Sham	5,30 ± 2,72	4,91 ± 1,82	6,10 ± 1,78	5,14 ± 0,95	5,77 ± 1,53	5,84 ± 1,94	4,89 ± 2,31	5,69 ± 1,99
Aβ-1	4,90 ± 1,99	4,20 ± 1,59	4,64 ± 1,70	5,09 ± 2,15	5,00 ± 1,65	5,20 ± 2,32	4,21 ± 1,49	5,14 ± 1,52
Aβ-2	4,05 ± 1,70	3,97 ± 1,83	4,56 ± 1,50	4,34 ± 1,68	4,84 ± 2,28	5,26 ± 1,23	3,81 ± 1,36	5,06 ± 1,95
Aβ-3	3,61 ± 1,64	3,51 ± 1,66	4,47 ± 1,95	4,24 ± 1,63	4,84 ± 1,34	4,85 ± 1,65	3,65 ± 1,57	4,88 ± 1,60
Aβ-4	**3,56 ± 2,03**	**3,51 ± 1,63**	**4,13 ± 1,35**	**4,02 ± 1,76**	**4,48 ± 2,02**	**4,76± 2,23**	**3,29 ± 0,98**	**4,72 ± 1,88**
Aβ-5	**3,56 ± 1,17**	**3,52 ± 2,57**	**3,67 ± 2,67**	**3,94 ± 2,02**	**4,41 ± 1,05**	**4,64 ± 1,04**	**3,19 ± 1,67**	**4,86 ± 1,72**
Aβ-6	**3,00 ± 1,50**	**3,05 ± 1,51**	**3,57 ± 2,06**	**3,52 ± 1,33**	**4,57 ± 1,76**	**4,53 ± 1,95**	**3,25 ± 1,52**	**3,85 ± 2,00**
Aβ-7	**2,50 ± 0,79**	**2,73 ± 0,84**	**3,17 ± 1,25**	**2,94 ± 1,71**	**4,13 ± 1,65**	**4,03 ± 1,44**	**3,10 ± 1,52**	**3,83 ± 1,78**

Tab. 4

Tab. 4. Means and standard deviations of maximum gamma band ITC values of AEPs in Sham and Aβ groups. Bold indicates a significant difference compared to the Sham (Greenhouse-Geisser corrected, p < 0.001).

Groups	FrA1	FrA2	PtA1	PtA2	TeA1	TeA2	V1	V2
Sham	0,22 ± 0,05	0,21 ± 0,05	0,27 ± 0,05	0,25 ± 0,03	0,22 ± 0,09	0,21 ± 0,09	0,24 ± 0,10	0,24 ± 0,09
Aβ-1	0,22 ± 0,07	0,21 ± 0,05	0,24 ± 0,09	0,24 ± 0,10	0,22 ± 0,07	0,22 ± 0,06	0,21 ± 0,04	0,23 ± 0,06
Aβ-2	0,19 ± 0,06	0,20 ± 0,07	0,23 ± 0,07	0,22 ± 0,05	0,20 ± 0,04	0,20 ± 0,06	0,20 ± 0,05	0,22 ± 0,06
Aβ-3	0,16 ± 0,04	0,17 ± 0,05	0,22 ± 0,06	0,20 ± 0,07	0,20 ± 0,07	0,19 ± 0,06	0,20 ± 0,03	0,20 ± 0,04
Aβ-4	**0,17 ± 0,04**	**0,16 ± 0,08**	**0,20 ± 0,06**	**0,20 ± 0,06**	**0,19 ± 0,05**	**0,19 ± 0,04**	**0,20 ± 0,06**	**0,20 ± 0,06**
Aβ-5	**0,16 ± 0,06**	**0,15 ± 0,08**	**0,19 ± 0,05**	**0,18 ± 0,07**	**0,18 ± 0,05**	**0,18 ± 0,03**	**0,16 ± 0,03**	**0,20 ± 0,04**
Aβ-6	**0,15 ± 0,06**	**0,14 ± 0,05**	**0,17 ± 0,07**	**0,17 ± 0,07**	**0,17 ± 0,06**	**0,17 ± 0,04**	**0,16 ± 0,08**	**0,18 ± 0,06**
Aβ-7	**0,14 ± 0,04**	**0,14 ± 0,04**	**0,16 ± 0,04**	**0,14 ± 0,08**	**0,17 ± 0,04**	**0,16 ± 0,06**	**0,14 ± 0,05**	**0,16 ± 0,05**

Tab. 5

Tab. 5. Means and standard deviations of filtered (28-48 Hz) auditory evoked gamma oscillatory responses values in Sham and Aβ groups. Bold indicates a significant difference in Aβ groups compared with the Sham group (Greenhouse-Geisser corrected, p < 0.001). Also, the asterisk indicates a significant difference in Aβ-7 group compared to the other Aβ groups (Greenhouse-Geisser corrected, p < 0.001).

Groups	FrA1	FrA2	PtA1	PtA2	TeA1	TeA2	V1	V2
Sham	25,98±10,74	23,96±11,56	34,87±11,05	34,95±15,47	37,95±8,17	38,71±9,39	21,88±13,50	23,37±13,68
Aβ-1	**11,71±4,16**	**13,15±4,77**	**14,15±5,57**	**16,38±7,83**	**13,79±5,28**	**12,04±5,44**	**9,24±3,47**	**8,52±3,62**
Aβ-2	**14,33±3,65**	**14,34±3,81**	**16,00±4,83**	**16,37±2,54**	**16,27±3,61**	**15,81±3,28**	**11,88±5,71**	**9,51±4,44**
Aβ-3	**10,86±4,59**	**12,05±4,93**	**13,30±3,55**	**16,16±5,82**	**13,86±5,04**	**14,13±4,84**	**10,86±3,26**	**11,26±5,84**
Aβ-4	**8,50±4,24**	**8,36±4,40**	**10,21±5,09**	**11,19±5,46**	**10,99±2,90**	**14,05±3,72**	**9,64±3,98**	**10,71±3,62**
Aβ-5	**11,32±4,47**	**10,19±3,10**	**14,93±4,43**	**13,57±4,26**	**14,09±5,18**	**14,60±4,57**	**9,82±3,67**	**9,09±2,80**
Aβ-6	**8,10±3,78**	**8,53±4,67**	**12,38±6,03**	**13,82±7,14**	**16,13±6,51**	**15,60±6,48**	**11,27±3,79**	**12,79±5,42**
Aβ-7	**7,84±4,51**	**5,30±2,19**	**12,24±4,34**	**8,04±3,23**	**6,92±2,53***	**5,52±2,02***	**5,78±2,72**	**4,29±1,13**

AKDENIZ UNIVERSITY MEDICAL SCHOOL
Department of Biophysics

Dear Sir/Madam

Enclosed please find the paper (ADVMS-D-19-00290R1) on the subject of "**Auditory evoked potentials might have the potential to serve as early indicators related to amyloid beta peptide toxicity**" to be published in the Journal of "*Advances in Medical Sciences*". We have necessary changes in the article according to the comments of reviewers. We think it is more satisfactory than the last version. Also, we confirm that all figures and tables which use in this paper are original.

In any case, if a question arises on the paper, please do not hesitate to contact me as soon as possible.

Thank you very much for your consideration.

With best regards

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