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Protective role of rosmarinic acid on amyloid beta 42-induced echoic memory decline: Implication of oxidative stress and cholinergic impairment

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Abstract: Abstract
In the present study, we examined whether rosmarinic acid (RA) reverses amyloid β (Aβ) induced reductions in antioxidant defense, lipid peroxidation, cholinergic damage as well as the central auditory deficits. For this purpose, Wistar rats were randomly divided into four groups; Sham(S), Sham+RA (SR), Aβ42 peptide (Aβ) and Aβ42 peptide+RA (AβR) groups. Rat model of Alzheimer was established by bilateral injection of Aβ42 peptide (2,2 nmol/10µl) into the lateral ventricles. RA (50mg/kg, daily) was administered orally by gavage for 14 days after intracerebroventricular injection. At the end of the experimental period, we recorded the auditory event related potentials (AERPs) and mismatch negativity (MMN) response to assess auditory functions followed by histological and biochemical analysis. Aβ42 injection led to a significant increase in the levels of thiobarbituric acid reactive substances (TBARS) and 4-Hydroxy-2-nonenal (4-HNE) but decreased the activity of antioxidant enzymes (SOD, CAT, GSH-Px) and glutathione levels. Moreover, Aβ42 injection resulted in a reduction in the acetylcholine content and acetylcholine esterase activity. RA treatment prevented the observed alterations in the AβR group. Furthermore, RA attenuated the increased Aβ staining and astrocyte activation. We also found that Aβ42 injection decreased the MMN response and theta power/coherence of AERPs, suggesting an impairing effect on auditory discrimination and echoic memory processes. RA treatment reversed the Aβ42 related alterations in AERP parameters. In conclusion, our study demonstrates that RA prevented Aβ-induced antioxidant-oxidant imbalance and cholinergic damage, which may contribute to the improvement of neural network dynamics of auditory processes in this rat model.

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Dear Sir/Madam

Enclosed please find the revised paper on the subject of "Protective role of rosmarinic acid on amyloid beta 42-induced echoic memory decline: implication of oxidative stress and cholinergic impairment" to be published in the Journal of "Neuroscience". I certainly hope, that the paper would satisfy all the requirements for the Journal in order to be published. I have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience. We believed that this is an original work as far as the research concern never the less. All authors have approved this manuscript to be published in the "Neuroscience". I have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience.

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

Thank you for your consideration.

Sincerely,

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Highlights

- Aβ42 affects antioxidant-oxidant balance and cholinergic system.
- Aβ42 causes deficit in network dynamics of auditory system.
- Rosmarinic acid reverses the Aβ42 induced alterations in auditory functions.
- Cholinergic enhancement via rosmarinic acid might be useful in AD.
- Rosmarinic acid might be efficient in AD treatment with its multiple bioactivities.

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Protective role of rosmarinic acid on amyloid beta 42-induced echoic memory decline: implication of oxidative stress and cholinergic impairment

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Abstract

In the present study, we examined whether rosmarinic acid (RA) reverses amyloid β (Aβ) induced reductions in antioxidant defense, lipid peroxidation, cholinergic damage as well as the central auditory deficits. For this purpose, Wistar rats were randomly divided into four groups; Sham(S), Sham+RA (SR), Aβ42 peptide (Aβ) and Aβ42 peptide+RA (AβR) groups. Rat model of Alzheimer was established by bilateral injection of Aβ42 peptide (2,2 nmol/10μl) into the lateral ventricles. RA (50mg/kg, daily) was administered orally by gavage for 14 days after intracerebroventricular injection. At the end of the experimental period, we recorded the auditory event related potentials (AERPs) and mismatch negativity (MMN) response to assess auditory functions followed by histological and biochemical analysis. Aβ42 injection led to a significant increase in the levels of thiobarbituric acid reactive substances (TBARS) and 4-Hydroxy-2-nonenal (4-HNE) but decreased the activity of antioxidant enzymes (SOD, CAT, GSH-Px) and glutathione levels. Moreover, Aβ42 injection resulted in a reduction in the acetylcholine content and acetylcholine esterase activity. RA treatment prevented the observed alterations in the AβR group. Furthermore, RA attenuated the increased Aβ staining and astrocyte activation. We also found that Aβ42 injection decreased the MMN response and theta power/coherence of AERPs, suggesting an impairing effect on auditory discrimination and echoic memory processes. RA treatment reversed the Aβ42 related alterations in AERP parameters. In conclusion, our study demonstrates that RA prevented Aβ-induced antioxidant-oxidant imbalance and cholinergic damage, which may contribute to the improvement of neural network dynamics of auditory processes in this rat model.

Key words: Amyloid β, Rosmarinic acid, Lipid peroxidation, Antioxidant system, Cholinergic system, Auditory event related potential.

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disease which is the most commonly recognized cause of dementia in the aging population (Brookmeyer et al., 2007. All forms of AD are characterized by extraneuronal deposits of amyloid β (Aβ) peptide and the intraneuronal accumulation of hyperphosphorylated tau {Blennow, 2006 #2310). Although the cause of AD is still unknown, previous studies highlighted the role of Aβ peptides in the pathogenesis of the disease. In the amyloid cascade hypothesis, it is assumed that increased Aβ accumulation has a pivotal role in the pathological cascades causing to increased oxidative stress, activation of astrocytes, neuronal dysfunction and loss, ultimately leading to cognitive dysfunction (Butterfield, 2002, Palop and Mucke, 2010). It is known that cholinergic neurons are more vulnerable to Aβ toxicity. Although the role of cholinergic dysfunction in the etiology of AD is controversial, observed cholinergic abnormalities in AD patients and well-established role of cholinergic system in cognition support the idea that the reduced cholinergic neurotransmission is implicated in AD pathogenesis (Perry et al., 1981, Whitehouse et al., 1982). In a previous report, it was proposed that the reduction of cholinergic activity may weaken the compensatory capacity of the brain for secondary insults (Craig et al., 2011). Thus, the mechanisms by which Aβ42 induces cholinergic dysfunction may involve the production of toxic mediators such as reactive oxygen intermediates.

Accumulating evidence indicates that increased oxidative stress plays an important role in the etiology and progression of AD. It is known that Aβ peptide, as a mediator of oxidative stress, causes lipid peroxidation, protein oxidation, and thereby contribute to the pathological condition (Lauderback et al., 2001, Butterfield, 2002). Oxidative stress is caused by the imbalance between the rate of oxidants production and the level of antioxidants. Because of low activity of

antioxidant defense system and high content of polyunsaturated fatty acids, brain is susceptible to oxidative stress more than the other organs. Studies showing decreased glutathione content and altered enzyme activities in several brain regions of AD patients indicate that antioxidant defense system is affected in the AD (Gsell et al., 1995, Kim et al., 2003). In this context, the use of natural compounds containing a high concentration of antioxidants in the prevention and treatment of AD is a promising strategy. Naturally occurring antioxidants such as polyphenolic compounds have received great attention because they are perceived as safe and multifunctional compounds to treat the neurodegenerative diseases. Previous studies revealed that rosmarinic acid (RA) is one of the most potent antioxidant among the hydroxycinnamic group of polyphenols (Soobrattee et al., 2005) which was associated with improved antioxidant potency, including enhanced glutathione content and activity of antioxidant enzymes in vitro (Fallarini et al., 2009, Chkhikvishvili et al., 2013). In addition, RA also possesses multiple biological activities such as anti-inflammatory and anti-amyloidogenic effects (Makina et al., 2002, Ono et al., 2012). A few studies (El Omri et al., 2010, Mushtaq et al., 2014) showed the modulatory effect of RA containing extracts on cholinergic system but the effect of RA on Aβ42 induced cholinergic deficit is largely unknown.

Several auditory tests such as the dichotic digits and phoneme discrimination were used to examine the auditory functions in patients and these studies concluded that central auditory processing impairs in individuals with AD (Iliadou and Kaprinis, 2003, Gates et al., 2011). Furthermore, earlier studies suggested that there is an association between cognitive decline and central auditory dysfunction (Gates et al., 2011). As it is an early symptom of the disease, it is important to examine how Aβ contributes to the auditory impairment. To do so, we examined the altered brain activity in the auditory event related potentials (AERPs) and relevant biochemical changes. Electrophysiological correlate of auditory sensory memory is the AERP component

named mismatch negativity (MMN). MMN is related to involuntary attention and reflects the brain's capacity to discriminate sounds regardless of the individual's attentional and behavioral capacity. Initially described by Näätänen et al. (1978) (Naatanen et al., 1978), MMN is a cortically evoked potential that is detectable when a change occurs in the middle of a sequence of repeated acoustic stimuli. On the other hand, MMN (MMN like AERP) have also been recorded in various nonhuman animal species including primates and rats (Javitt et al., 1992, Eriksson and Villa, 2005). In awake and anesthetized rats, the MMN has been found to occur in the latency range of 30–250 ms (Ruusuvirta et al., 1998, Nakamura et al., 2011). So, MMN may be a good indicator of auditory dysfunction in Aβ induced rat model of AD. To date, effects of RA on AERPs in the Aβ induced AD animal model have not yet been investigated. Therefore, the goal of the present study was to investigate possible protective effect of RA on neuronal toxicity of Aβ in the auditory system which was evaluated through AERPs, neurochemical and histopathological analyses. In order to evaluate the relationship between oxidative cell injury, cholinergic markers and differences in AERP parameters, thiobarbituric acid reactive substances (TBARS), 4-hydroxy-2-nonenal (4-HNE) levels, acetylcholine (ACh) content and acetylcholine esterase (AChE) activity of the brain tissue were determined in the present research. Additionally, to examine the antioxidant defense system, glutathione (GSH) content, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities were determined.

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Materials and Methods

Animals and treatment

All animal experiments were approved by the Institutional Animal Care and Use Committee at Akdeniz University Medical School. Male albino Wistar rats aged 3 months, weighing 250 to 300 g were housed in stainless steel cages in groups of 4 rats per cage and given food and water *ad libitum*. Animals were maintained at 12 h light-dark cycles and a constant temperature of 23 ± 1°C at all times. Animals were divided into four groups (n=10 each group): (1) sham operated plus physiological saline treatment (S); (2) sham operated plus RA treatment (SR); (3) Aβ42 (2.2 nmol/10 µl) i.c.v. (intracerebroventricularly) injection plus physiological saline treatment (Aβ);(4) Aβ42 (2.2 nmol/10 µl) i.c.v. injection plus RA treatment (50 mg/kg, i.p.) (AβR). Drug treatment began one day after the surgical procedure. Treatment occurred once a day for 15 days. RA was purchased from Carbosynth (Carbosynth, San Diego, CA, USA). The purity of RA was >98%. RA has a molecular formula of $C_{18}H_{16}O_8$, and molecular weight of 360.31 g/mol. 50 mg of RA were dissolved in 1 ml 0.9% saline solution. The dosage was chosen according to the results of our earlier study (Gok et al., 2015). Aβ42 (Sigma Chemicals, USA, product no: A9810) was diluted in sterile normal saline to a final concentration of 1 µg/µl. To obtain the aggregated form of Aβ42, the peptide solution was placed in an incubator at 37 °C for 72 h.

Animal preparation

The rat model of AD was established as described previously (Li et al., 2010). Rats were anesthetized with a combination of ketamine (80 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) and then placed in a standard stereotaxic apparatus. A middle sagittal incision was made in the scalp and was sterilized using standard procedures. Bilateral holes were drilled in the skull using a

dental drill over the lateral ventricles. Injection coordinates were chosen according to the atlas of Paxinos and Watson (AP: −0.8mm, ML: ±1.4mm, DV: −4.0 mm). Rats in Aβ and AβR groups were injected with 2,2 nmol/10μl Aβ42 at a rate of 0.5μl/min. The syringe was removed 5 min after the injection. The S and SR groups received sterile normal saline. After surgery, the scalp was sutured, and sulfamethoxazole was sprinkled on the wound to prevent infection. In addition, penicillin (40,000 U) was injected intramuscularly into the gluteus, once a day for 3 days.

Biochemical Measurements

After the experimental period, animals were sacrificed by an overdose injection of anesthetic agent the next day at the same time interval (9:00 am and 2:00 pm). Brain tissues were collected and immediately stored in ice-cold buffer. The isolated brain tissues were homogenized separately for TBARS/CAT (50mM potassium phosphate, pH 7.0, containing 1mM EDTA), GSH-Px (50mM Tris-HCL, containing 5mM EDTA, and 1mM DTT, pH 7.5), SOD (2-5 ml HEPES buffer),4-HNE/GSH (5-10 ml PBS, pH 7.4)and ACh/AChE (20 mM sodium phosphate buffer, pH 7.4).

Thiobarbituric acid reactive substances assay

A part of sonicated samples were centrifuged at 14,000 g for 10 min at 4 ^{o}C in an eppendorf microcentrifuge (Biofuge 15R, Heraeus Sepatech, Osterode, Germany). The supernatant of centrifuged samples was used for the assay of TBARS measurements. Levels of TBARS were measured by a fluorimetric method described by Wasowicz et al. (1993) (Wasowicz et al., 1993), using 1,1,3,3-tetraethoxypropane as a standard. The results are reported as μmol/g protein.

4-Hydroxy-2-nonenal assay

The quantity of HNE adduct in protein samples was detected by 4-HNE Adduct ELISA kit (Cat# STA-838; OxiSelect; Cell Biolabs Inc., San Diego, CA) according to the manufacturer's instruction. In this kit, Bovine serum albumin (BSA) standards or protein samples (10 µg/ml) are adsorbed onto a 96-well plate for 2 hrs at 37oC. The HNE-protein adducts present in the sample and standard are probed with an anti-HNE antibody, followed by a HRP conjugated secondary antibody. The HNE-protein adducts content in an unknown sample is determined by comparing with a standard curve that is prepared from predetermined HNE-BSA standards. The quantity of HNE adduct was expressed in µg per mg protein.

Measurement of superoxide dismutase activity

The SOD activity was measured using assay kit (Cat #706002; Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instruction. This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. SOD activity was expressed in unit per µg protein.

Measurement of catalase activity

The CAT activity was measured using assay kit (Cat #707002; Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instruction. This kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based, briefly, on the reaction of enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. One unit of CAT activity was defined as the amount of enzyme that

causes the formation of 1.0 nmol of formaldehyde per minute at 25°C. CAT activity was expressed in unit per µg protein.

Measurement of glutathione peroxidase activity

The GSH-Px activity was measured using assay kit (Cat #703102; Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instruction. The measurement of GSH-Px activity is based on the principle of a coupled reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after reduction of hydroperoxide by GSH-Px is recycled to its reduced state by GR in the presence of nicotinamide-adenine dinucleotide phosphate (NADPH). The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm. One unit of GSH-Px was defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25oC. GSH-Px activity was expressed in unit per mg protein.

Measurement of total glutathione

The GSH levels were measured by a commercially available GSH assay kit (Cat #703002; Cayman Chemical, Ann Arbor, MI, USA). Supernatants were deproteinated in 10% metaphosphoric acid (Sigma Aldrich, Steinheim, Switzerland). The GSSG was reduced to GSH by GSH reductase in the assay cocktail of the kit containing 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), glucose-6-phosphate dehydrogenase, GSH reductase, Nicotinamide adenine dinucleotidephosphate (NADP+) and glucose-6-phosphate. The sulfhydryl group of GSH reacts with DTNB to give a yellow colored 5-thio-2-nitrobenzoic acid (TNB) which is measured at an absorbance of 405 nm. The values of total GSH for each sample were calculated from their respective slopes using a GSSG or GSH standard curve. Total GSH levels were expressed in mmol per mg protein.

Measurement of acetylcholine level

The ACh level was measured using an Amplex® Red Acetylcholine/Acetylcholinesterase Assay Kit (A-12217; Invitrogen, USA). According to the manufacturer's instructions, reactions were initiated by adding 100 μl of the working solution, containing 400 μM Amplex Red reagent, 2 U/ml horseradish peroxidase (HRP), 0.2 U/ml choline oxidase, and 1 U/ml acetylcholinesterase, to each microplate well containing 100 μl of the standard or test sample. Each reaction was incubated for 1 h at room temperature with plate agitation and protection from light. Absorbance was then measured using a microplate reader (Molecular Devices, USA) at a wavelength of 563 nm. Acetylcholine levels were calculated from a standard curve and expressed as mmol/g protein.

Measurement of acetylcholinesterase activity

The AChE activity was also measured using an Amplex® Red Acetylcholine/Acetylcholinesterase Assay Kit (A-12217; Invitrogen). A working solution, containing 400 μM Amplex Red reagent, 2 U/ml HRP, 0.2 U/ml choline oxidase, and 100 μM acetylcholine, was used for AChE activity measurement. Absorbance was measured using a microplate reader (Molecular Devices, USA) at a wavelength of 590 nm.

Determination of protein

Protein concentrations in brain tissues were spectrophotometrically measured (Shimadzu RF-5500, Kyoto, Japan) by a protein assay reagent kit (Pierce, Rockford, IL) via a modified Bradford method. Bovine serum albumin was used as a standard.

Pathologic examination and immunohistochemistry

Brain tissue was fixed with formaldehyde for pathologic examination. Formalin fixed brain tissues were sliced in coronal sections and whole brain were processed. Paraffin embedded tissues were cut in 4μm thick sections and were stained with Hemotoxylin&Eosin. They were examined blindly on light microscopy by one pathologist. The sections containing auditory cortex and hippocampus were selected for immunhistochemical analyzes. All sections were examined on a Zeiss-Axioplan, microscope (Zeiss, Oberkochen, Germany).

Immunohistochemical staining was performed by using Ventana Benchmark LT (Ventana Medical Systems, Tucson, AZ, USA) with its standard protocol. Briefly, selected paraffin embedded blocks was cut into 4-μm-thick sections. Sections were incubated at 60℃ for 5 min and then incubated with polyclonal rabbit anti-rat Aβ antibody (1:1000) (Cell Signaling, Beverly, MA, USA) and GFAP antibody (1:250) (Abcam, ab7260, USA) using a closed-system automated immunohistochemical staining device (Ventana, Roche, United States). Aβ42 levels and GFAP expression were quantified using Image J (1.48v) software (NIH, USA), which confers semi-quantitatively analysis of immunohistochemical staining.

MMN recordings and analysis

At the end of the experiment, rats were anesthetized with intraperitoneal injections of urethane (1.2 g/kg, Sigma-Aldrich, St Louis, Missouri, USA) and prepared for MMN recordings. Briefly the head of the anesthetized animal was attached to the standard stereotaxic frame and six screws were placed in the skull. Recording electrodes were placed bilaterally on frontal and auditory cortex and reference and ground electrodes were placed on cerebellar skull. The tip of a stainless steel wire was positioned to the surface of the dura on the basis of online-recorded potentials to click stimuli. After the surgery, the ear bars were removed. The anesthetized animal was then

moved into a sound-attenuated recording room. Mean background noise level of the recording room measured 46 dB with a sound level meter (Testo 816 Sound Level Meter, Germany).

AERPs were recorded using the oddball condition, experimental set-up adapted and modified from Astikainen et al. (Astikainen et al., 2011). In the oddball condition for auditory stimuli, frequencies of standard and deviant tones were 2000 and 2500 Hz, respectively. Deviant tones were pseudorandomized to occur at a 10% probability (900 standard tones, 100 deviant tones) in a sequence of standard tones. In the oddball, two separate stimulus blocks were presented with interstimulus intervals of 375 and 600 ms. The tones were ordered pseudorandomly in their series with the restriction that there were no less than two standards between consecutive deviants. The duration of the 85-dB tones was 50 ms and the tones were presented through a loudspeaker at a distance of 15 cm from the ears of the animal.

Electroencephalogram 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). Data recorded during oddball condition were filtered (0.1–150 Hz) and baseline corrected (the average amplitude of a 50-ms period preceding stimulus onset) (Brainvision Analyzer, Brain Products, Munich, Germany). For both ISI value, the following averaged curves were computed for each animal and then for all groups of animals: Standard before deviant (StbD) (AERPs to standard tones preceding deviant tones), Deviant (Dev) (AERPs to all deviant tones during the oddball paradigm). The peak-to peak amplitudes and latencies of the P1, N1, P2 and N2 components of the AERPs were determined for each rat from the averaged StbD and Dev curves within the time period of 0–350 from stimulus onset.

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MMN response was obtained by subtracting the averaged curves corresponding to the StbD stimulus from the averaged curves obtained in response to the Dev stimulus. The MMN was identified as the wave of negative polarity and with approximate latency of 50-200 ms post-stimulus. Because the MMN was defined as the part of the AERP wave where there is a significant difference between deviant and standard responses, AERP curves in this time window were submitted to paired sample t-test and repeated measures of ANOVA (for stimulus effect) to verify the MMN response. Amplitude measurements were analyzed and calculated by placing one of the reference cursors on the negative polarity point and the other cursor on the positive point previous to MMN between 50–200 ms.

Power spectrum analysis

The epochs (0 -375 ms, 0-600 ms) of each rat were averaged for each electrode location and then the digital FFT-based power spectrum analysis was performed using a Hanning window with 10% taper length with brainvision analyzer. The standard frequency band of interest was theta (4–8 Hz). At this point, the theta frequency range was chosen for investigating the Aβ induced effects on AERPs, since the role of theta oscillations in discrimination process and MMN generation was identified in the previous studies (Ko et al., 2012, Kaser et al., 2013). The maximum individual theta frequency value for each rat was included, for the purpose of statistical analysis, as the maximum individual theta frequency value of that rat.

Coherence

Coherence was calculated with BrainVision Analyzer using the following equation:

$$Coh(c_1,c_2)(f)=|CS(c_1,c_2)(f)|^2/(|CS(c_1,c_2)(f)||CS(c_1,c_2)(f)|)$$

where $CS(c_1,c_2)(f) = \Sigma\, c_{1,\,i}(f)\, c_{2,\,i}(f)$. For the theta frequency band (4–8 Hz), coherence values were computed from the cross spectrum for the target stimuli for intrahemispheric and inter-hemispheric electrode pairs.

Statistical analysis

The statistical analysis of the obtained data was performed by SPSS 18.0 (SPSS, Chicago, IL, USA) software for Windows. Statistical comparisons between groups for all biochemical parameters were performed by using one way ANOVA and post-hoc Bonferroni test. Statistical comparisons between groups for GFAP and Aβ staining were performed by using Kruskal-Wallis one-way analysis of variance and Mann-Whitney U test.

The latencies and peak to peak amplitudes of P1, N1, P2 and N2 components of the AERPs and theta power were analyzed by means of a repeated measure ANOVA including the between subject factor groups (S, R, Aβ, AβR) and the within subject factor locations (F_{left}, F_{right}, $AC_{left,}$ AC_{right}), stimulus (Dev, StbD) (Bonferroni post hoc test). Greenhouse–Geisser corrected p-values are reported. The comparisons of P2N2 responses between the two stimulus levels for each group were made using the paired sample t-test. MMN amplitudes were examined via repeated measure ANOVA included between subject factor groups (S, R, Aβ, AβR), and within-subject factor locations (F_{left}, F_{right}, $AC_{left,}$ AC_{right}). Post hoc comparisons were analyzed with Bonferroni test. The coherence values were analyzed by a repeated measures ANOVAs with one between subject factor(S, R, Aβ, AβR), and two within-subject factors (ISI: 375, 600; electrode pairs)(Bonferroni post hoc test). Separate repeated measures ANOVAs were conducted for the intrahemispheric and interhemispheric electrode pairs for theta frequency band.

Results

Thiobarbituric acid reactive substances levels

Lipid peroxidation was measured as the amount of TBARS. Mean TBARS values of the brain tissues of sham and experimental groups are given in Fig. 1A. There was a statistically significant difference between groups [$F_{3.36} = 12.83$, $p < 0.001$]. Brain TBARS levels were significantly increased in the Aβ (0,33 ± 0,02 μmol/g protein) group versus the S (0,26 ± 0,01 μmol/g protein) and SR (0,19 ± 0,02 μmol/g protein) groups ($p < 0.01$ for all comparisons). RA treated-AβR (0,21 ± 0,02 μmol/g protein) group had lower TBARS levels versus the Aβ group ($p < 0.001$). RA treatment alone had no impact on TBARS level in the SR group when compared to S group.

4-Hydroxy-2-nonenal levels

Mean values of brain 4-HNE levels are given in Fig. 1B. There was a statistically significant difference in 4-HNE levels between groups [$F_{3.36} = 17.25$, $p < 0.001$]. Brain 4-HNE levels were significantly increased in the Aβ (1,85 ± 0,05 μg/mg protein) group with respect to S (1,42 ± 0,04 μg/mg protein) and SR (1,35 ± 0,06 μg/mg protein) groups, and significantly decreased in the AβR (1,36 ± 0,06 μg/mg protein) group versus the Aβ group ($p < 0.001$ for all comparisons).

Superoxide dismutase activity

SOD activities of the brain tissues of all experimental groups are shown in Fig. 2A. There was a statistically significant difference between groups [$F_{3.36} = 23.19$, $p < 0.001$]. SOD activity was significantly attenuated in the Aβ (0,12 ± 0,02 U/μg protein) group in comparison with S (0,19 ± 0,02 U/μg protein) and SR (0,25 ± 0,01 U/μg protein) groups ($p < 0.001$ for all comparisons). SOD activity was significantly increased in the AβR (0,29 ± 0,02 U/μg protein) group with

respect to Aβ group ($p < 0.001$). Although a slight increase was seen in the SOD activity of SR group, this increment did not reached the significant level.

Catalase activity

CAT activities in the brain tissues of all experimental groups are presented in Fig. 2B. There was a statistically significant difference between groups [$F_{3.36} = 46.27$, $p < 0.001$]. CAT activity was significantly decreased in the Aβ (0,13 ± 0,02 U/μg protein) group as compared to S (0,32 ± 0,01 U/μg protein) and SR (0,39 ± 0,03 U/μg protein) groups ($p < 0.001$ for all comparisons). The activity of CAT was significantly increased in the RA treated AβR (0,45 ± 0,01 U/μg protein) group versus the Aβ and also S group ($p < 0.001$). RA treatment alone was slightly increased the CAT activity in the SR group but this increment did not reached the significant level.

Glutathione peroxidase activity

GSH-Px activities of the brain tissues of sham and experimental groups are shown in Fig. 2C. There was a statistically significant difference between groups [$F_{3.36} = 33.55$, $p < 0.001$]. GSH-Px activity was significantly decreased in the Aβ (0,66 ± 0,04 U/mg protein) group versus S (1,00 ± 0,08 U/mg protein) and SR (1,12 ± 0,07 U/mg protein) groups ($p < 0.001$ for all comparisons). The activity of GSH-Px was significantly increased in AβR (1,21 ± 0,05 U/mg protein) group versus the Aβ ($p < 0.001$) and also S ($p < 0.01$) and SR groups ($p < 0.05$). RA treatment alone slightly increased the GSH-Px activity in the SR group but this increment did not reached the significance level.

Glutathione Levels

Mean values of brain GSH level are given in Fig. 2D. There was a statistically significant difference in GSH levels between groups [$F_{3.36} = 106.61$, $p < 0.001$]. Brain GSH levels were significantly decreased in the Aβ (1,03 ± 0,05 mmol/mg protein) group with respect to S (2,61 ± 0,09 mmol/mg protein) and SR (2,85 ± 0,12 mmol/mg protein) groups. Although GSH level was significantly increased in the AβR (1,49 ± 0,03 mmol/mg protein) in comparison to the Aβ group ($p < 0.01$), it did not reach to sham level.

Acetylcholine levels

Mean values of brain ACh levels are given in Fig. 3A. There was a statistically significant difference in ACh levels between groups [$F_{3.36} = 65.51$, $p < 0.001$]. Brain ACh levels were significantly decreased in the Aβ (4,99 ± 0,38 mmol/g protein) group versus S (8,88 ± 0,42 mmol/g protein) and SR (11,60 ± 0,43 mmol/g protein) groups. The decrement in the ACh level was reversed by the RA administration and also increased versus the S group in the AβR (12,79 ± 0,50 mmol/g protein) group ($p < 0.001$ for all comparisons). RA treatment alone was slightly increased the ACh level in the SR group but this increment did not reached the significance level.

Acetylcholine esterase activity

AChE activities in the brain tissues of all groups are shown in Fig. 3B. There was a statistically significant difference between groups [$F_{3.36} = 23.29$, $p < 0.001$]. AChE activity was significantly attenuated in the Aβ (1,70 ± 0,12 U/mg protein) group with respect to S (3,02 ± 0,22 U/mg protein) and SR (3,90 ± 0,19 U/mg protein) groups ($p < 0.05$ for all comparisons). The AChE activity was significantly increased in AβR (4,10 ± 0,40 U/mg protein) group versus the Aβ and S groups ($p < 0.001$). RA treatment alone was slightly increased the AChE activity in the SR group but this increment did not reached the significance level.

Amyloid β and GFAP immunostaining

Immunohistochemical analysis of Aβ deposits and GFAP are shown in Fig. 4. There was a very little intraneuronal Aβ immunolabeling localized in hippocampal areas and auditory cortex of S and SR groups (Fig. 4A). On the other hand, intense Aβ immunoreactivity was clearly observed in the cytoplasm of hippocampal and auditory cortical areas in the Aβ group. Aβ immunostaining in the AβR group was not as intense as in the Aβ group. Quantitative analysis of Aβ stained sections revealed that there was a significant difference among groups (HC: $H(3) = 13.78$, $p<0.01$; AC: $H(3) = 11.27$, $p<0.05$) (Fig. 4B). Both in hippocampus and auditory cortex, higher Aβ immunoreactivity and percentage of stained area were detected in the Aβ group versus the S and SR groups ($p < 0.05$ for all comparisons). RA treatment significantly decreased Aβ staining in the AβR group versus the Aβ group (Fig. 4B) ($p < 0.05$ for all comparisons).

Normal astrocyte structure was observed in the hippocampal and auditory cortical areas of the S and SR groups (Fig. 4C). Intense GFAP immunostaining were detected in the hippocampus and auditory cortex areas of Aβ group. It was due to the increment in the number of astrocytes that have long, thick branching and distended cell body as an indicator of astrogliosis. RA significantly inhibited the Aβ42 induced astrocytic reaction in hippocampus and auditory cortex (Fig. 4C).Quantitative analysis of GFAP stained sections revealed that there was a significant difference among groups (HC: $H(3) =12.79$, $p<0.01$; AC: $H(3) = 9.55$, $p<0.05$) (Fig. 4D). Higher GFAP immunoreactivity and % stained area in the hippocampus and auditory cortex of Aβ group were decreased significantly attenuated in the AβR group (Fig. 4D) ($p < 0.05$ for all comparisons).

Auditory event related potentials and MMN response

Fig. 5 illustrates the P1, N1, P2 and N2 components of AERPs to StbD and Dev tones in the two oddball conditions for all experimental groups. Difference waveforms (DW) obtained by subtracting standard responses from deviant ones are also shown in the same figure (Fig. 5). Measurements were made on two positive and two negative potentials which were seen in all of the groups. In the present study, there was no main group effect on latencies of AERP components for both ISI values.

Mean±SEM of peak-to-peak amplitudes of AERP components (P1N1, N1P2, P2N2) in response to StbD and Dev tones for 375 ms ISI are shown in Table 1. The analysis of amplitudes demonstrated that there was no main group effect for 375 ms ISI value for all AERP components. Mean±SEM of peak-to-peak amplitudes of AERP components (P1N1, N1P2, P2N2) in response to StbD and Dev tones for 600 ms ISI are shown in Table 2. When we examined the amplitudes for 600 ms ISI value, repeated ANOVA indicated a significant group effect [$F_{3.36} = 1714.09$, $p < 0.001$]. Also, there was significant stimulus effect for N1P2 [Stim: $F_{1.36} = 683.57$, $p < 0.001$] and P2N2 amplitudes [Stim: $F_{1.36} = 763.92$, $p < 0.001$]. Post-hoc comparisons showed that N1P2 response to Dev stimulus was significantly smaller than N1P2 response to StbD stimulus in S and SR groups over the right frontal and auditory regions ($p < 0.001$ for all comparisons). For the Aβ group, we didn't observe the decrement in N1P2 amplitude to Dev stimulus and any amplitude difference between N1P2 responses to Dev and StbD stimulus ($p > 0.05$). RA treatment in the AβR group was reconstituted the decrement pattern and amplitude difference between N1P2 responses to Dev and StbD stimulus in right frontal and auditory regions ($p < 0.001$ for all comparisons).

In a reverse manner, P2N2 response to Dev stimulus was significantly larger versus P2N2 response to StbD stimulus in S and SR groups over the right frontal and auditory regions (p <

0.001 for all comparisons). This amplitude increment to Dev response was not observed in the Aβ group ($p > 0.05$). RA treatment was reconstituted the increment pattern of P2N2 response in the AβR rats. In parallel, between group comparisons indicated that Aβ group had higher N1P2 amplitude than S and SR groups for Dev stimulus over right frontal ($p < 0.001$) and auditory regions ($p < 0.05$ for all comparisons). N1P2 amplitude significantly decreased and returned to sham level in the AβR group versus the Aβ group in the same electrode regions ($p < 0.01$ for all comparisons). In a similar manner, for Dev stimulus, P2N2 amplitude was decreased in the Aβ group versus the S and SR groups and significantly increased in the AβR group with respect to the Aβ group over the same regions ($p < 0.01$ for all comparisons).

There was no main group effect on MMN responses for 375 ms ISI value. When we examined the MMN amplitudes for 600 ms ISI value, repeated ANOVA indicated a significant group effect [$F_{3.36}= 22.17$, $p < 0.001$]. Post-hoc comparisons showed that MMN response was significantly decreased in the Aβ group in comparison to S and SR groups over right and auditory regions ($p < 0.01$ for all comparisons). MMN amplitude was significantly reversed to sham level by rosmarinic acid administration in the AβR group.

Mean±SEM of theta power values of AERPs in response to StbD and Dev tones for 375 and 600 ms ISI values are shown in Table 3. In the present study, we did not observe any difference between groups in terms of theta power values for 375 ms ISI value. However, there was significant stimulus effect [$F_{1.36} = 115.68$, $p < 0.001$] for theta power. Post-hoc analysis showed that theta power was significantly increased in response to Dev stimulus versus the StbD stimulus over the left frontal and auditory regions in all groups. For 600 ms ISI value, we observed a significant main effect of group on theta power of AERPs [$F_{3.36}= 10.98$; $p < 0.001$]. Moreover, there was also significant stimulus effect [$F_{1.36} = 145.96$, $p < 0.001$] independent of groups. Post-

hoc analysis showed that there was no significant difference between theta power for Dev and StbD stimulus in Aβ group. For Dev stimulus, theta power was significantly decreased in the Aβ group in comparison to S and SR groups over right and auditory regions ($p < 0.01$ for all comparisons). Theta power value was significantly reversed to sham level by RA administration in the AβR group.

Mean±SEM of theta coherence values of AERPs in response to StbD and Dev tones for 375 and 600 ms ISI values are shown in Table 4. In the analysis of interhemispheric coherence differences, the ANOVA on theta coherence revealed a significant group effect [$F_{3.36}= 25.05$, $p < 0.01$]. Moreover, there were significant stimulus [$F_{1.36}= 10.73$, $p < 0.01$] and [$F_{1.36}= 408.26$, $p < 0.001$] electrode pairs effects. However, there was no significant ISI effect [$F_{1.36}= 1.04$, $p > 0.05$]. These results indicate an increase in theta coherence over auditory electrode pairs for Dev stimulus in both ISI values. Post-hoc comparisons showed that theta coherence was significantly lower for Aβ group than S and SR groups at the auditory electrode pair for Dev stimulus in both ISI values ($p < 0.001$ for all comparisons). RA administration was significantly increased the decreased theta coherence values over auditory electrode pairs in the AβR group compared to Aβ group. The analysis of intrahemispheric coherence revealed no significant main and interaction effect for any of these parameters.

Discussion

In the present study, we used a rat model of AD based on the i.c.v. application of Aβ42 to induce the changes similar to AD pathology. Similar to transgenic models, exogenous Aβ administration does not reproduce the full complexity of the human AD pathology. However, there is an impressive amount of evidence showing that exogenous administration of various Aβ peptides have been reported to induce considerable grade of neurodegeneration and glial

activation, as well as a reduction in levels of ACh, proximal to Aβ deposits (LaFerla et al., 1995, Pepeu et al., 1996).

Oxidative stress which resulted from an overproduction of reactive oxygen species (ROS) or from a reduction in antioxidant defenses has been suggested to be a primer initiating factor or contributor to the neurodegeneration seen in AD (Filipcik et al., 2006, Ansari and Scheff, 2010). In this respect, TBARS and 4-HNE levels were considered in the current study since they have been widely used as markers of lipid peroxidation caused by oxidant stress. Our data have shown that Aβ42 injection significantly increased TBARS and 4-HNE levels in rats compared with sham group. Our finding is in agreement with earlier studies indicated that Aβ peptides promotes oxidative stress and directly responsible for free-radical damage to neuronal membrane systems that lead to subsequent increase in lipid peroxidation (Butterfield, 2002, Palop and Mucke, 2010). Hence, our findings suggest that oxidative stress plays a central role in the pathogenesis of AD, because the formation of toxic lipid peroxidation causes peroxidative damage of membrane structure and changes in associated enzymes, receptors and physiological functions, that may ultimately result in disturbances in neuronal functions. On the other hand, we determined SOD, CAT, GSH-Px activities and GSH levels to examine the antioxidant defense system. We found a significant reduction in SOD/CAT and GSH/GSH-Px system that is in accordance with the findings of some animal and human studies (Kaminsky and Kosenko, 2008, Padurariu et al., 2010, Puertas et al., 2012). However, other studies in the literature reported contradictory findings such as no change in GSH-Px (Marcus et al., 1998, Sultana et al., 2008), a significant increase (Lovell et al., 1995)or no change (Gsell et al., 1995) in SOD or increased CAT activity (Lovell et al., 1995) in AD. Nevertheless, collectively these findings suggest that Aβ42 peptide induce a general weakening in the antioxidant defense system which fails either due to overproduction of free radicals or decrement in activities of scavenging enzymes. Regarding the

observed alterations in brain of AD rats, previous studies as well as the present findings support the view that GSH level is a consistent marker for antioxidant status in Aβ treated animal models. Because GSH is major antioxidant of the brain, its reduction leads to a situation where the rate of ROS production exceeds the antioxidant ability and thereby generating a situation that favors oxidative stress.

Extensive literature from human and animal studies supports the notion that cholinergic dysfunction in the central nervous system is another important factor that contributes to the molecular changes associated with AD (Whitehouse et al., 1982, Bartus and Emerich, 1999, Auld et al., 2002). Within this context, we examined the ACh level and AChE activity to determine the effect of Aβ peptide on cholinergic system. Consistent with earlier studies indicating a relationship between cholinergic dysfunction and degree of Aβ deposition (Tran et al., 2002, Parihar and Hemnani, 2004, Jicha and Carr, 2010), brain ACh level and AChE activity showed a marked decrease in the Aβ injected rats versus the other groups. Several mechanisms can explain the Aβ induced cholinergic damage. First, Aβ peptide interacts with some membrane receptors that facilitate its internalization and thereby mediate its toxic effects directly in cholinergic cells. Secondly, Aβ peptides activate several kinases such as mitogen-activated protein kinase and glycogen synthase kinase-3beta which are known to be involved in tau phosphorylation and thereby lead to neuronal death via disruption of the cytoskeletal network. Thirdly, long-term exposure to Aβ peptide induces an increase in choline transmission that in turn leads to choline depletion. As a consequence, usage of membrane phosphatidylcholine to synthesize ACh causes disruption of membrane turnover and damage. Last, taking into account the peroxidative effect of Aβ obtained in the present research as well as the previous studies showing the role of oxidative stress in both kinase activation and cytotoxicity, it is conceivable to suggest that Aβ induced lipid

peroxidation may play an important role in the observed changes in cholinergic parameters. In addition, considering the neuroprotective (neurogenesis, neurotrophic factors, changes in dendritic branching role of ACh in the brain, reduction in ACh mediated compensatory mechanisms may be an important factor in the enhancement of oxidative damage to the membranes as we observed in the Aβ group. Consistent with this view, in a previous report it was proposed that cholinergic depletion leads to a dampening in the ability of the brain to compensate for secondary insults and augment the degenerative processes (Craig et al., 2011).

It is well known that reactive astrogliosis prominently takes place in AD pathology as an inflammatory response and astrogliosis detected in the cortex and hippocampal areas of patients with AD (Boekhoorn et al., 2006). In accordance with this previous report (Li et al., 2010), we found a significant enhancement of GFAP immunoreactivity in hippocampus and auditory cortex of Aβ42 injected rats. This increase can be explained partly by hypertrophy and upregulation of the GFAP expression in reactive astrocytes, which is considered to play a role in the events involved in Aβ neurotoxicity.

There has been a growing interest in the potential use of natural polyphenols since recent evidence has indicated that they have multiple biological activities which can be relevant to reverse degenerative processes in many ways. Therefore, we investigated the protective effects of RA, a naturally occurring hydroxylated polyphenolic compound, on Aβ induced neurotoxicity. The current results clearly demonstrated that RA attenuated lipid peroxidation induced by Aβ. Moreover, RA increased the SOD, CAT, GSH-Px activity and GSH level, suggesting that it was also potentiated scavenge of ROS possibly by improving the activity of the antioxidant system.

Substantial evidence proposed that AD causes synaptic dysfunction early in the disease process, disrupting communication within cholinergic neural circuits that are important for

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memory and other cognitive functions. To increase levels of ACh by suppressing AChE activity is one of the main therapeutic approaches in AD. But, in the present study, we found that RA increased not only cortical ACh levels but also AChE activity in the AβR group. Therefore, we propose that RA has a potentiating effect on cholinergic system and consequently caused an increment in AChE activity. Our present data are compatible with the report of El Omri (El Omri et al., 2010) showing that RA might potentiate cholinergic system by increasing both ACh level and AChE activity. However, our findings contradict with some biochemical and animal studies which have reported anticholinesterase activity of RA and/or RA containing extracts (Mushtaq et al., 2014, Soodi et al., 2014, Vladimir-Knezevic et al., 2014). This contradiction could be attributed to the application and dose differences.

Our findings together with previous observations strongly suggest that RA may induce neuroprotection and may provide memory improvement against Aβ42 peptide. Moreover, RA administration prevented the decrease of cholinergic markers in the brain of Aβ42 injected rats. To the best of our knowledge, this is the first study that examined the effect of RA on cholinergic markers in Aβ induced neurotoxicity. Therefore, it is likely that the RA exerts protective effects against oxidative stress via preventing alterations in cholinergic activity. In addition, RA showed anti-amyloidogenic effect with decreased Aβ immunostaining and also decreased GFAP over expression in parallel with its antioxidant effects. Reduction in Aβ accumulation accompanied by increases in antioxidant defense and cholinergic markers may attenuate astrocyte activation. Consequently, the preventive effects of RA such as reducing lipid peroxidation and oxidative stress might be associated with the cholinergic property exhibited by this polyphenol.

In the current study, we recorded AERPs and analyzed the MMN response to determine the effects of RA to the central auditory dysfunction which seen in an early stage of AD.

Consistent with previous studies (Ruusuvirta et al., 1998, Eriksson and Villa, 2005, Astikainen et al., 2011), in comparison to standard tones, deviant tones elicited MMN response in shorter ISI over the left frontal and auditory regions in all experimental groups. Our data demonstrated that Aβ42 peptide induced alterations did not interfere with the short-term echoic memory processes. In the right frontal area we did not observe any significant MMN response in sham group rats. In the light of these data, we suggest that the MMN generation might be left dominant over frontal areas. Our data is in parallel with a dynamic causal modeling study (Garrido et al., 2009)which showed that intrinsic connections within bilateral primary auditory cortices and extrinsically connections from right secondary auditory cortex to right frontal cortex were involved in MMN generation network. In contrast, human studies indicated right hemisphere dominance for tones (Levanen et al., 1996) and left hemisphere dominance for phonemes in deviant detection paradigms (Naatanen et al., 1997, Tervaniemi et al., 2000). On the other hand, our data clearly indicated that MMN responses in longer ISI were decreased in Aβ group in comparison with the sham group over the mentioned areas. It is plausible that increasing ISI value increases the echoic memory load in the system. Therefore, it could be expected that MMN response in AD patients were diminished in longer ISI assessed by study of Pekkonen (Pekkonen et al., 2001). Moreover, based on the elevated levels of TBARS and 4-HNE, we can conclude that lipid peroxidation might have a role in the altered auditory processing for long ISI value in this group. Additionally, decreased cholinergic markers in Aβ42 administered rats might affect neuronal dynamics in the generation of MMN.

The power of the theta band oscillation showed a significant increase with the Dev stimulus. This significant increase is consistent with previous studies indicating that MMN is related with an increase in theta power in deviant tones (Ko et al., 2012, Kaser et al., 2013). Increased frontal

theta response has been associated with information processing and error monitoring (Basar-Eroglu et al., 1992). In parallel with amplitude results, this increment in theta power was diminished in Aβ group. As mentioned above, theta oscillations play an important role in the generation of MMN component. Therefore, it could be concluded that Aβ peptide interfered with the theta activity in the network. So, reduced MMN amplitude and theta spectral power of Aβ injected rats in longer ISI suggest that even do their auditory discrimination ability is intact, their echoic memory strength is impaired by Aβ exposure. Since AD patients have exhibited decreased theta activity in cognition, findings of this study support the view that reduced theta power might be the underlying oscillatory mechanism related to the decreased MMN amplitudes in patients. Considering the heterogeneous nature of theta rhythms, several mechanisms should be considered to explain these data. Previous reports indicate that cortical ACh modulate the general efficacy of the cortical processing of sensory stimulus or associational information by enhancing the influence of relevant stimulus for further processing (Sarter et al., 2005). For example, while ACh reduces the intracortical synaptic potentials, facilitates the thalamocortical input to the auditory cortex (Picciotto et al., 2012). In this way, ACh affects signal-to-noise ratio during sensory processing and modulates synchronization of neuronal networks. Thus, the functional role of ACh includes enhancement of both attention to sensory stimuli and encoding of memory for specific stimuli. Furthermore, relationship between cholinergic transmission and cortical theta oscillations was evidenced with the reduction in the event related theta power in the frontal cortex of medial septal lesioned animals. Also, neocortical brain slices had been shown to display oscillatory activity in the theta range when perfused with a cholinergic agonist (Lukatch and MacIver, 1997). Within this context, we suggest that Aβ induced cholinergic hypofunction may contribute to the observed decrement of theta power. Consequently, in agreement with earlier studies, our data strongly suggests that cholinergic inputs to the auditory cortex play an important

role in stimulus specific adaptation, plasticity and sensory memory (Pekkonen et al., 2001, Leach et al., 2013). Other possible mechanism might involve the impaired glutamatergic neurotransmission that is known to play a significant role in pathophysiology of AD. Previous research indicated that modulation of the glutamatergic system (via NMDA receptor modulation) was associated with MMN generation in auditory cortex (Javitt et al., 1996). Also, NMDA receptor blockade was shown to reduce theta frequency power (Lazarewicz et al., 2010) and phase locking (van Wingerden et al., 2012) in experimental studies.

In the current study we found significant decrease in theta coherence for the Dev stimulus that may reflect impaired cortical connectivity between cortical regions. Our results are in agreement with previous studies showing decreased coherence activity in AD evidenced with reduced resting states and evoked coherence in individuals with AD. Previously, it was proposed that impaired neural synchrony results from neocortical disconnection syndrome induced by degenerative processes (Delbeuck et al., 2003). Diffusion tensor imaging studies verified this view with identified disintegration of white matter tracts (Medina et al., 2006, Naggara et al., 2006). In addition, prominent accumulation of amyloid plaques was detected around cortical tracts previously (Pearson et al., 1984). Based on the foregoing findings, we concluded that the Aβ42 induced degenerative changes in the white matter might contribute to decrement of theta coherence. On the other hand, in line with our data, reduced cholinergic transmission with muscarinic antagonist, scopolamine, altered the interhemispheric functional connectivity during rest and photic stimulus (Ebert and Kirch, 1998). Based on the determined role of ACh in encoding novel information in hippocampus and also in sensory system, we suggest that reductions of ACh might play a role in the observed attenuation of theta synchronization during Dev stimulus. The evidence that cholinergic projections to the cerebral cortex are necessary for the beta and gamma

band synchronization (Rodriguez et al., 2004) is compatible with this hypothesis. Here, we demonstrated that 50 mg/kg RA treatment significantly reversed the Aβ induced changes in MMN response and theta dynamics. Moreover, in two different analyses we found that both Aβ induced decrement in theta power and auditory theta coherence were prevented by RA administration.

In conclusion, our results are consistent with previous findings that Aβ42 peptide induces lipid peroxidation, cholinergic dysfunction, and astroglial activation together with a general decrement in antioxidant capacity. Observed difference in AERPs and MMN only for longer ISI values indicates that Aβ42 effects sensory memory while not disturbing auditory perception. Furthermore, oral RA administration which prevent Aβ42 induced disruption of network dynamic underline MMN generation and thereby preserve echoic memory possible by increasing cholinergic tone and suppressing oxidative stress via attenuating lipid peroxidation and potentiating antioxidant defense. Thus, RA could be used as an efficient agent to prevent diverse pathological processes, which are also related with each other in different levels, with its multiple bioactivities.

Conflict of interest

There were no conflicts of interest reported by the authors of this paper.

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Legends of Figures

Fig. 1.

TBARS and 4-HNE levels of the brain tissues in sham and experimental groups. The results are presented as mean±SEM, n=10 for each group. (*significant vs. S group; $^{\xi}$significant vs. Aβ group)

Fig. 2.

SOD, CAT, GSH-Px activities and GSH levels of the brain tissues in sham and experimental groups. The results are presented as mean±SEM, n=10 for each group. (*significant vs. S group; $^{\xi}$significant vs. Aβ group)

Fig. 3.

ACh levels and AChE activities of the brain tissues in sham and experimental groups. The results are presented as mean±SEM, n=10 for each group. (*significant vs. S group; $^{\xi}$significant vs. Aβ group)

Fig. 4.

Immunohistochemical staining of Aβ peptide and GFAP protein in all experimental groups. Representative images of; Aβ peptide localization in the hippocampus and auditory cortex, GFAP localization in the hippocampus and auditory cortex. The scale bar is 20 µm. At the bottom panel: Quantitative analysis of; A: Aβ staining levels in the hippocampus, B: Aβ staining levels in the auditory cortex, C: GFAP staining levels in the hippocampus, D: GFAP staining levels in the

auditory cortex. Results are mean ± SEM, n=5 for each group. (*significant vs. S group; [ξ]significant vs. Aβ group)

Fig. 5.

Representative AERP responses to Standard (StbD) and deviants (Dev) tones in the oddball condition and the difference waves (DW) in the left auditory location for all groups. Two positive (P1,P2) and two negative (N1,N2).

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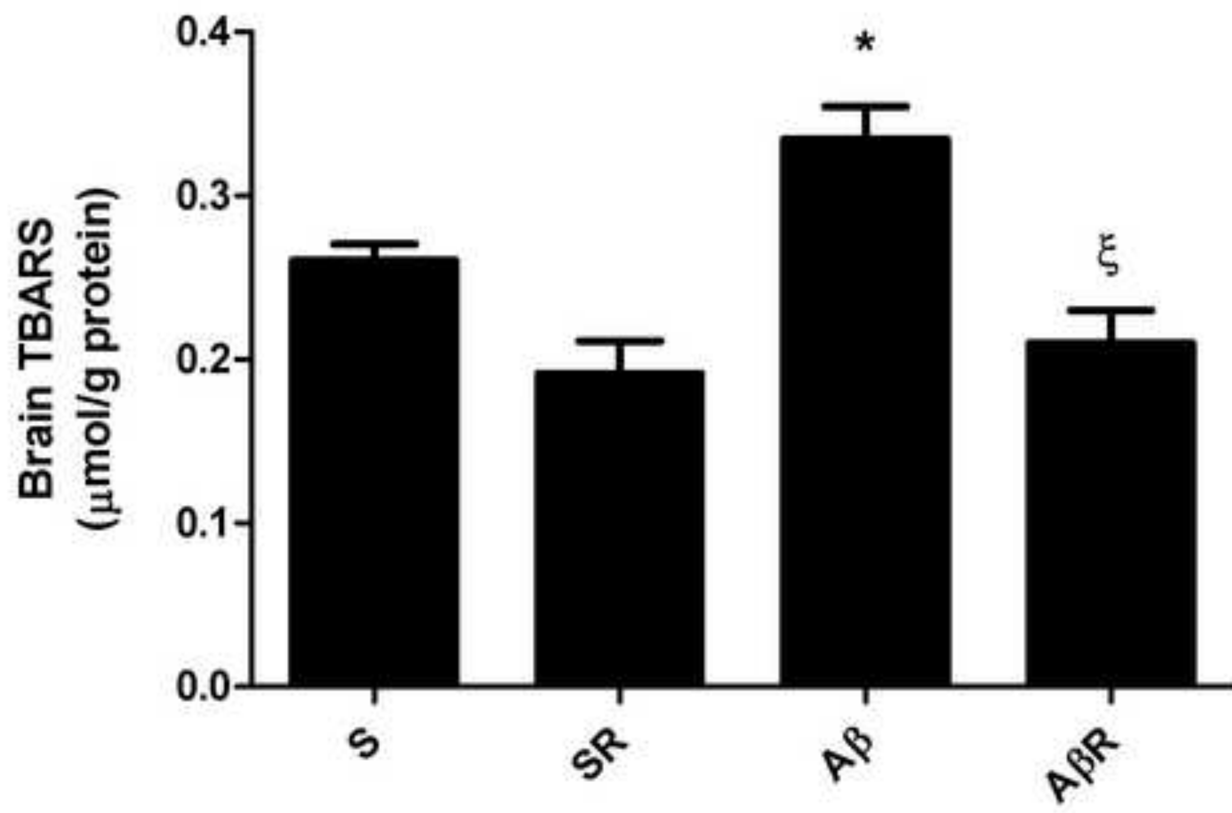

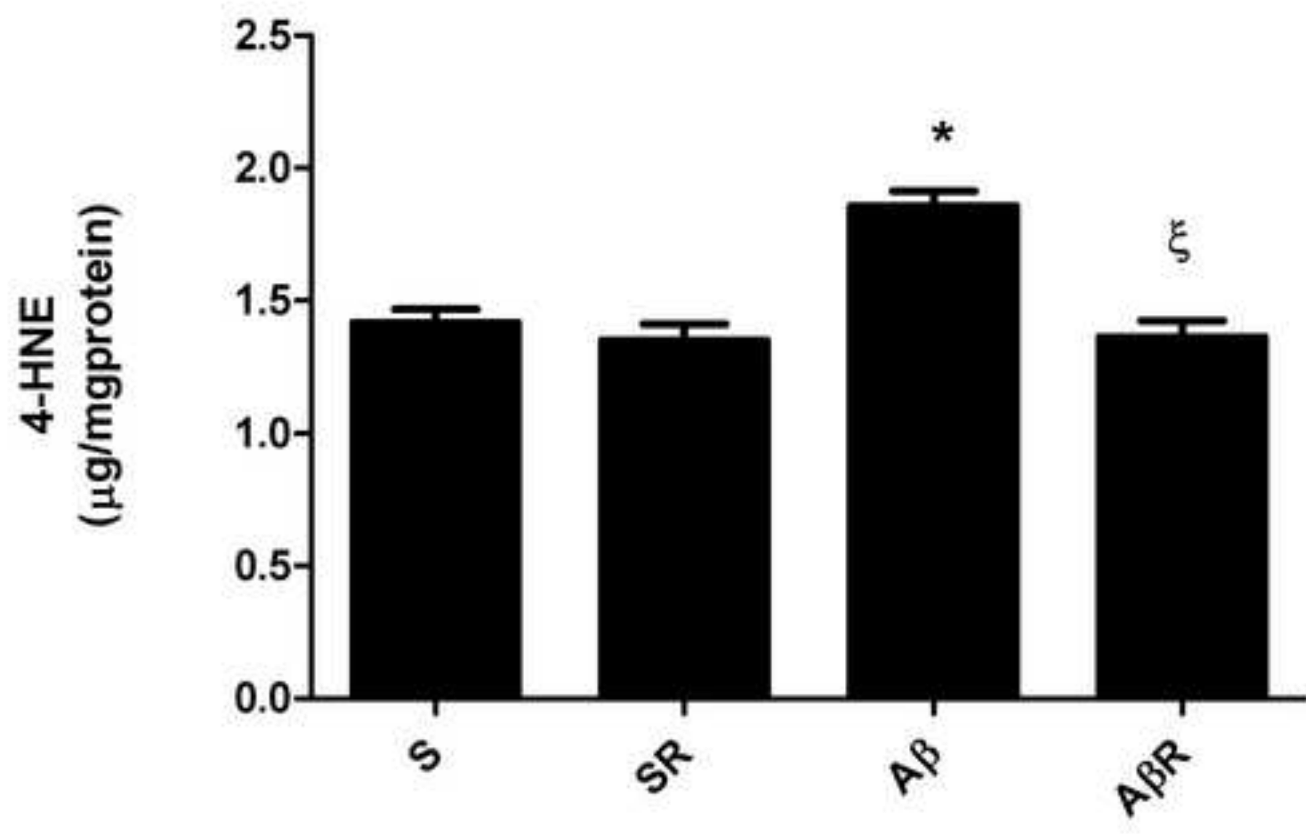

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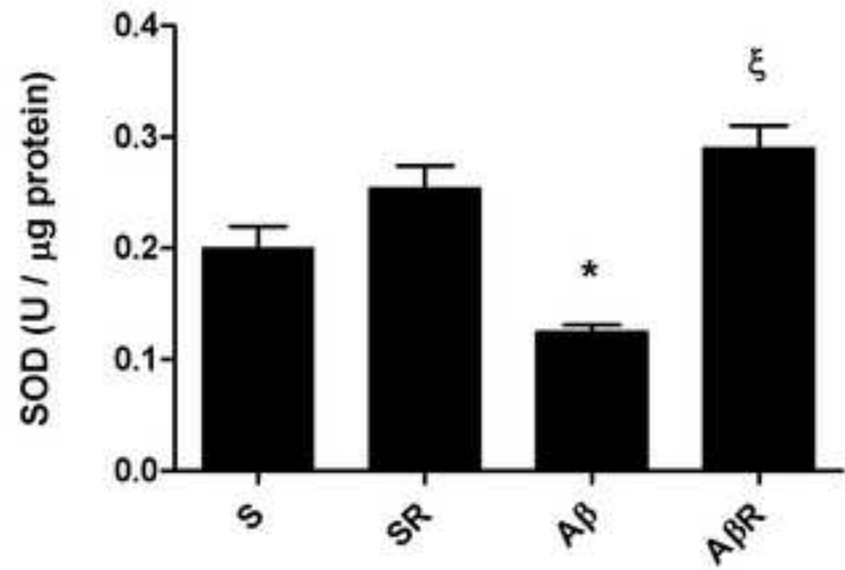

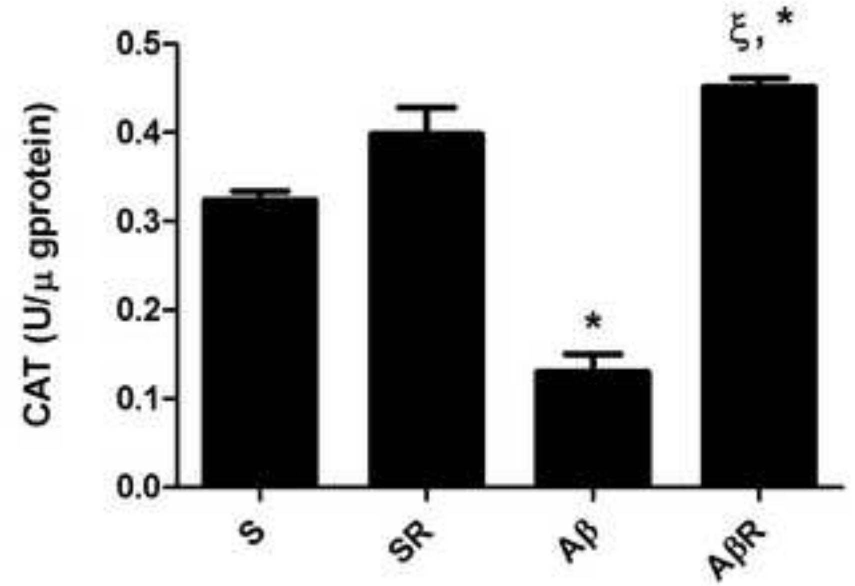

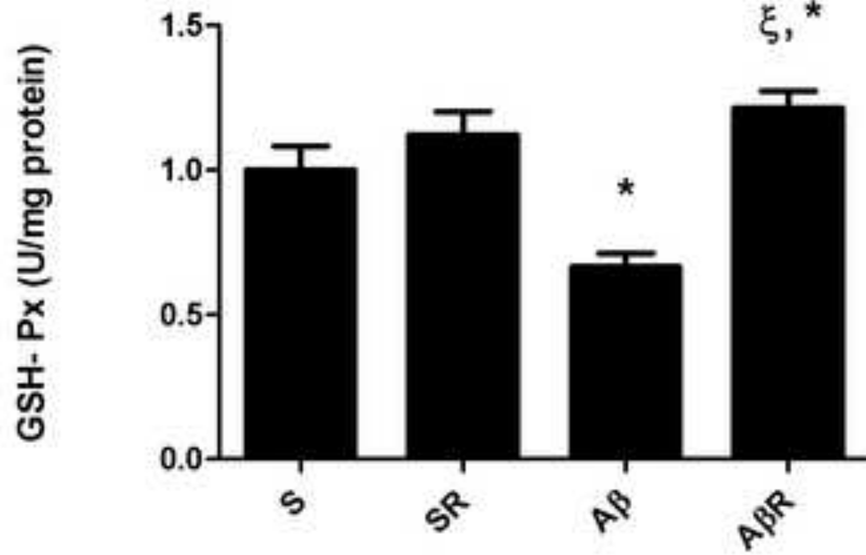

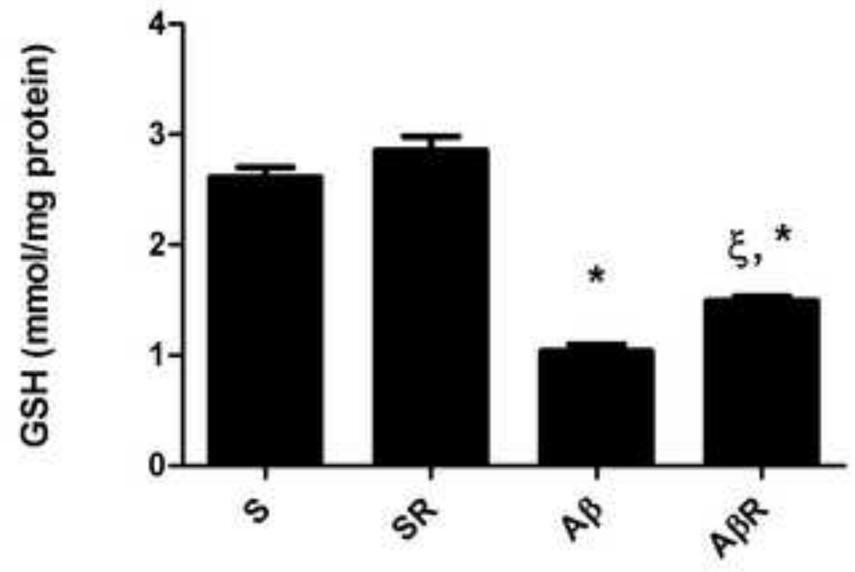

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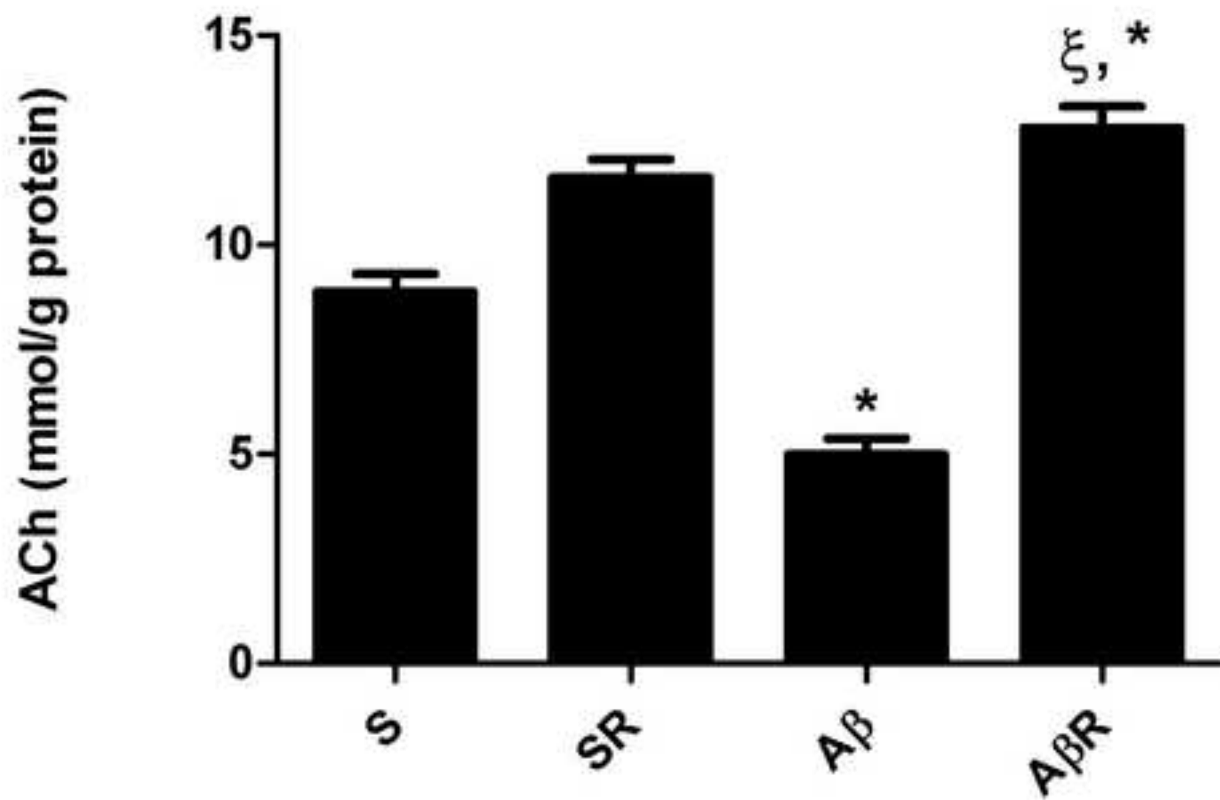

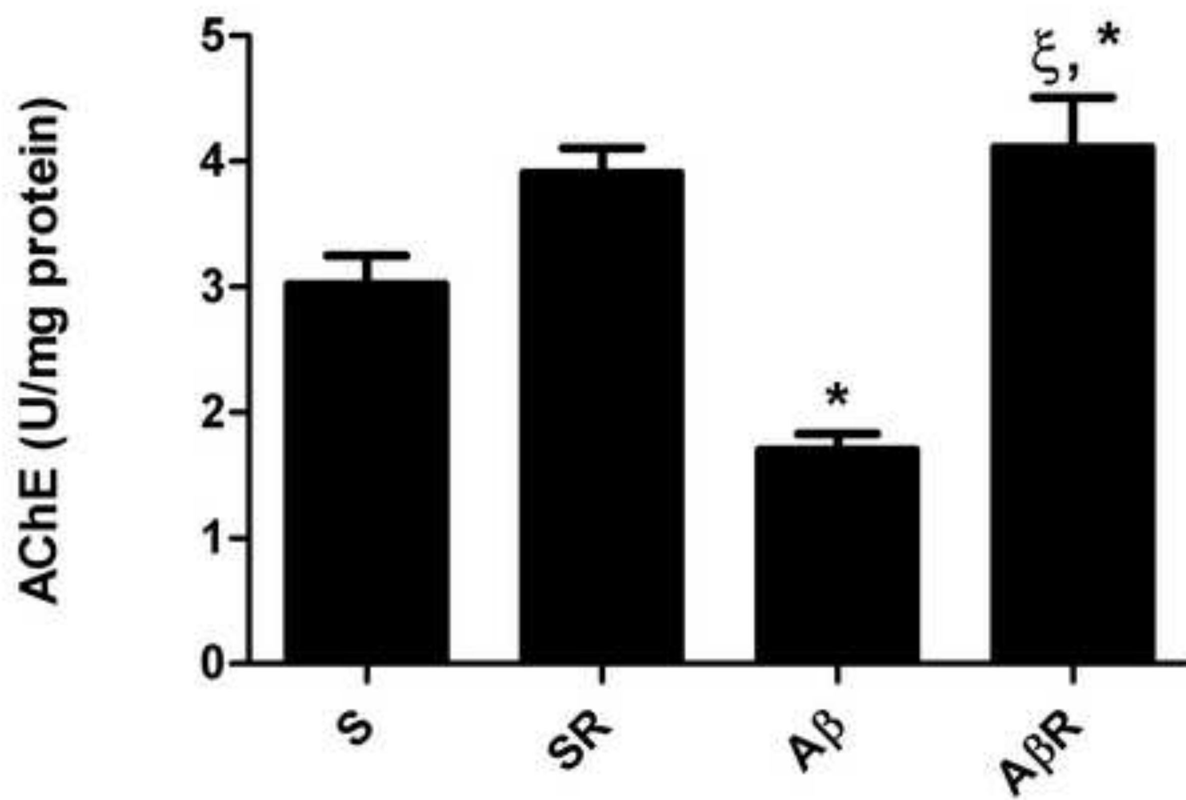

Figure 4
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Aβ

Hippocampus | Auditory Cortex

GFAP

Hippocampus | Auditory Cortex

S S S S
SR SR SR SR
Aβ Aβ Aβ Aβ
AβR AβR AβR AβR

Bottom panel

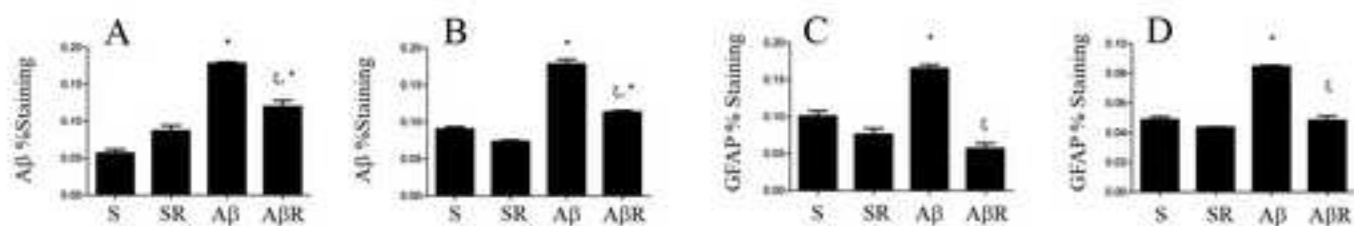

Figure 5

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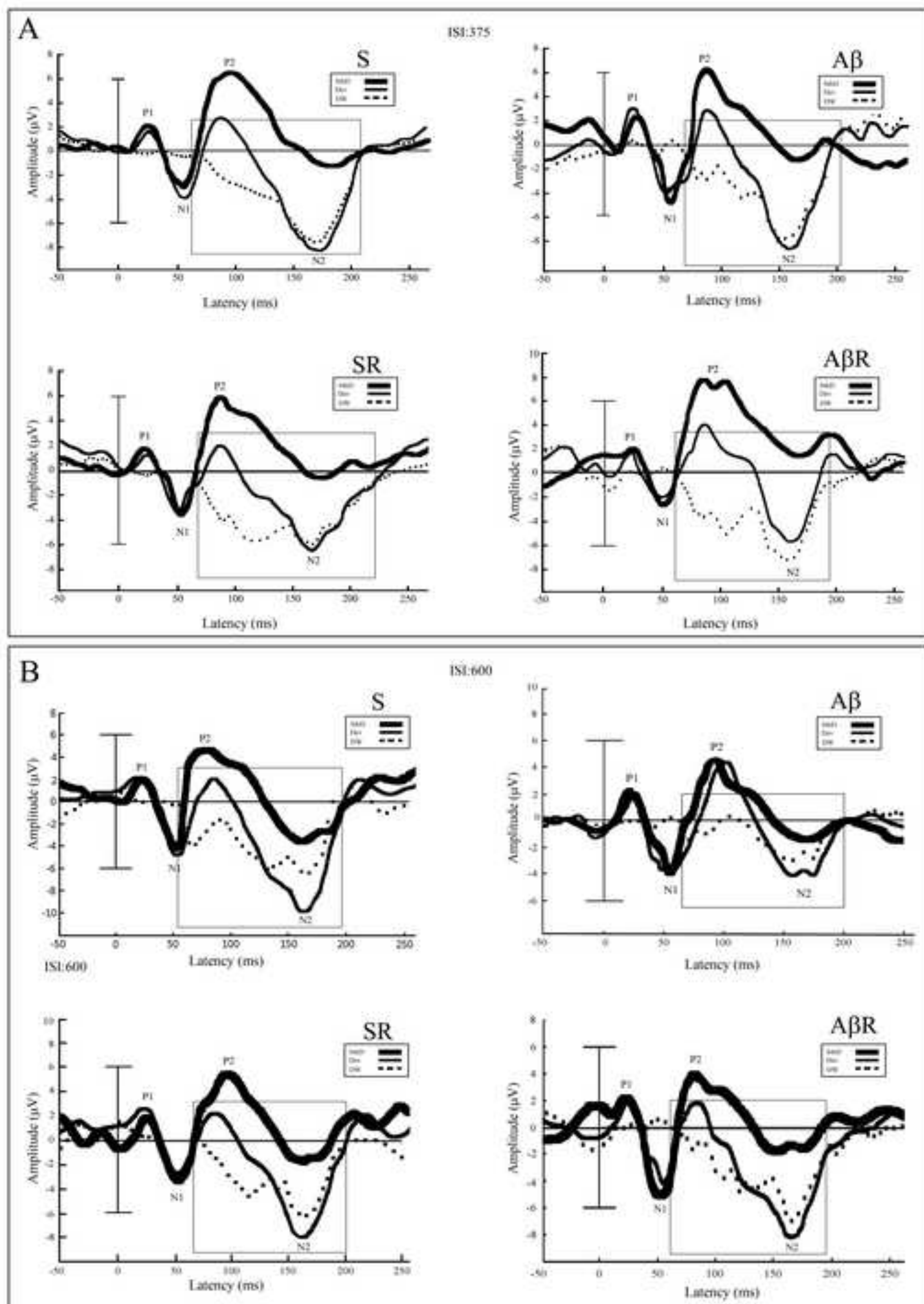

Tables

Table 1. The mean and standard errors of peak latencies of ERP components in response to Standard and the deviant tones for 375 ms ISI value in all experimental groups.

		P1N1		N1P2		P2N2	
	Groups	**StbD**	**Dev**	**StbD**	**Dev**	**StbD**	**Dev**
F_{right}	**S**	6,70 ± 0,76	5,36 ±0,39	7,14±0,45	7,07±0, 62	5,81±0,94	6,69±0,44
	SR	4,30 ± 0,48	4,96±0,50	6,99±0,58	4,63±0,55	4,22±0,48	5,66±0,35
	Aβ	4,83 ± 0,46	4,07±0,20	7,83±0,76	4,67±0,42	5,66±0,34	5,48±0,58
	AβR	4,80 ±0,57	4,46±0,62	7,40±0,39	4,39±0,46	5,51±0,60	5,78±0,39
F_{Left}	**S**	5,78±0,66	6,99±0,40	11,17±0,64	6,58±0,59 ψ	6,37±0,41	12,33±0,55 ψ
	SR	4,97±0,52	5,42±0,32	10,07±0,47	4,90±0,53 ψ	5,23±0,37	11,07±0,63 ψ
	Aβ	4,97±0,14	5,04±0,30	9,21±0,62	5,55±0,25 ψ	5,54±0,36	10,92±0,76 ψ
	AβR	4,45±0,52	4,64±0,45	10,33±0,32	4,67±0,54 ψ	5,46±0,59	10,35±0,36 ψ
AC_{right}	**S**	6,38±0,58	6,40±0,48	12,31±0,63	5,28±0,36 ψ	6,77±0,51	12,06±0,82 ψ
	SR	6,30±0,34	5,83±0,53	10,72±0,68	6,36±0,55 ψ	6,05±0,25	11,89±0,53 ψ
	Aβ	5,47±0,42	4,32±0,39	8,81±0,33	5,13±0,29 ψ	5,15±0,41	10,60±0,37 ψ
	AβR	5,34±0,44	5,78±0,52	9,34±0,51	5,38±0,25 ψ	5,17±0,27	9,51±0,70 ψ
AC_{Left}	**S**	5,20±0,37	5,99±0,48	12,75±0,64	5,76±0,25 ψ	6,36±0,33	13,03±0,60 ψ
	SR	5,90±0,41	5,85±0,61	11,51±0,61	5,10±0,46 ψ	5,93±0,34	10,63±0,26 ψ
	Aβ	6,40±0,59	6,30±0,59	11,07±0,60	5,04±0,37 ψ	5,60±0,19	11,55±0,63 ψ
	AβR	4,78±0,34	4,80±0,30	10,68±0,71	5,13±0,58 ψ	6,04±0,31	11,23±0,53 ψ

ψ : Significant difference in within group comparisons

Table 2. The mean and standard errors of peak latencies of AERP components in response to Standard and the deviant tones for 600 ms ISI value in all experimental groups.

		P1N1		N1P2		P2N2	
		StbD	**Dev**	**StbD**	**Dev**	**StbD**	**Dev**
F_{right}	**S**	5,23±0,58	5,36±0,29	7,28±0,60	5,80±0,23	5,91±0,25	5,21±0,19
	SR	4,37±0,14	4,87±0,33	8,51±0,25	6,07±0,16	5,61±0,32	5,71±0,24
	Aβ	4,86±0,59	6,66±0,43	7,25±0,65	5,04±0,33	6,07±0,67	5,97±0,45
	AβR	6,01±0,51	6,06±0,29	7,93±0,37	6,22±0,43	6,33±0,59	6,58±0,57
F_{left}	**S**	5,11±0,61	5,23±0,66	10,85±0,44	6,66±0,63 ψ	6,85±0,32	13,01±0,67 ψ
	SR	5,46±0,42	5,37±0,69	12,05±1,17	6,44±0,60 ψ	5,56±0,21	10,70±0,41 ψ
	Aβ	5,91±0,62	6,93±0,55	10,10±0,24	9,78±0,50 *	5,96±0,40	7,99±0,40 *
	AβR	6,43±0,37	6,41±0,47	11,17±0,59	7,01±0,51 ψ ξ	6,48±0,52	12,51±0,81 ψ ξ
AC_{right}	**S**	4,79±0,41	6,77±0,59	9,78±0,45	4,69±0,49 ψ	5,96±0,52	11,83±0,26 ψ
	SR	5,58±0,55	6,96±0,29	11,27±0,63	5,49±0,19 ψ	6,71±0,42	13,58±0,54 ψ
	Aβ	6,75±0,82	7,39±0,53	9,94±0,23	9,28±0,45 *	6,59±1,11	8,06±0,54 *
	AβR	6,70±0,45	6,02±0,41	10,48±0,47 ξ	5,56±0,28 ψ ξ	5,50±0,42	11,10±0,94 ψ ξ
AC_{left}	**S**	6,28±0,19	6,74±0,35	11,04±0,47	5,91±0,39 ψ	6,10±0,43	13,05±0,67 ψ
	SR	5,08±0,24	5,35±0,32	11,11±0,50	5,73±0,28 ψ	5,33±0,33	10,25±0,39 ψ
	Aβ	6,31±0,50	6,51±0,89	9,26±0,20	9,06±0,24 *	6,30±0,26	8,50±0,43 *
	AβR	6,33±0,21	6,12±0,39	10,50±0,68	6,01±0,64 ψ ξ	6,48±0,20	11,36±0,35 ψ ξ

Ψ : Significant difference in within group comparisons
* : Significant difference versus sham group
ξ : Significant difference versus Aβ group

Tablo 3. The mean and standard errors of theta power values of AERPs in response to standard and the deviant tones for 375 and 600 ms ISI values in all experimental groups.

Theta power (μV^2)

		ISI 375		ISI 600	
		StbD	**Dev**	**StbD**	**Dev**
Fright	**S**	5,70± 1,09	6,55± 2,45	4,40± 0,41	5,07± 0,47
	SR	5,64± 2,39	6,54± 1,05	4,70± 0,98	5,24± 0,81
	Aβ	6,00± 0,90	5,02± 0,49	3,90± 0,88	5,28± 1,55
	AβR	7,38± 1,92	6,08± 0,35	6,68± 0,67	7,32± 1,77
Fleft	**S**	6,69± 0,57	12,83± 1,12 ψ	4,57± 0,50	9,27± 0,32 ψ
	SR	6,74± 0,71	11,42± 0,65 ψ	4,18± 0,39	9,14± 0,27 ψ
	Aβ	6,14± 0,57	9,89± 0,28 ψ	5,84± 0,46	**5,40± 1,33 ***
	AβR	6,96± 0,45	9,62± 0,68 ψ	5,46± 0,48	12,10± 1,02 ψ ξ
ACright	**S**	7,89± 0,51	10,95± 0,59 ψ	4,90± 0,69	9,55± 0,39 ψ
	SR	7,30± 0,57	12,70± 0,56 ψ	5,16± 0,42	10,05± 0,42 ψ
	Aβ	6,54± 0,90	9,22± 0,96 ψ	6,62± 0,93	**5,32± 0,66 ***
	AβR	7,76± 0,32	12,52± 0,86 ψ	6,80± 0,46	11,52± 0,69 ψ ξ
ACleft	**S**	7,14± 0,61	12,09± 0,68 ψ	4,69± 0,46	9,90± 0,47
	SR	8,84± 0,51	13,78± 0,38 ψ	6,48± 0,58	10,40± 0,56 ψ
	Aβ	7,54± 0,49	12,08± 0,73 ψ	5,96± 1,03	**5,92± 0,35 ***
	AβR	8,28± 0,38	12,70± 0,59 ψ	5,74± 0,39	11,24± 0,41 ψ ξ

Ψ : Significant difference in within group comparisons

* : Significant difference versus sham group

ξ : Significant difference versus Aβ group

Table 4. The mean and standard errors of theta coherence values of AERPs in response to StbD and Dev tones for 375 and 600 ms ISI values in all experimental groups.

		F_{right}-F_{left}	AC_{right}-AC_{left}	F_{right}-AC_{right}	F_{right}-AC_{left}	F_{left}-AC_{right}	F_{left}-AC_{left}
ISI375 StbD	**S**	0,766± 0,028	0,618± 0,029	0,111± 0,013	0,085± 0,009	0,090± 0,008	0,084± 0,007
	SR	0,773± 0,023	0,616± 0,021	0,299± 0,098	0,125± 0,017	0,165± 0,026	0,150± 0,035
	Aβ	0,754± 0,021	0,624± 0,035	0,174± 0,022	0,118± 0,010	0,229± 0,017	0,166± 0,013
	AβR	0,746± 0,049	0,528± 0,043	0,194± 0,043	0,200± 0,010	0,240± 0,080	0,171± 0,017
ISI375 Dev	**S**	0,760± 0,015	0,596± 0,017	0,194± 0,029	0,132± 0,009	0,181± 0,023	0,143± 0,012
	SR	0,808± 0,033	0,616± 0,021	0,169± 0,020	0,180± 0,012	0,154± 0,015	0,166± 0,016
	Aβ	0,776± 0,022	**0,318± 0,026** *	0,115± 0,026	0,170± 0,033	0,114± 0,010	0,171± 0,049
	AβR	0,684± 0,032	**0,645± 0,021** ξ	0,164± 0,017	0,148± 0,017	0,230± 0,019	0,205± 0,013
ISI600 StbD	**S**	0,790± 0,042	0,646± 0,024	0,249± 0,104	0,151± 0,022	0,239± 0,082	0,190± 0,025
	SR	0,764± 0,018	0,556± 0,027	0,174± 0,028	0,162± 0,011	0,184± 0,023	0,183± 0,014
	Aβ	0,786± 0,030	0,624± 0,028	0,143± 0,042	0,103± 0,014	0,103± 0,021	0,141± 0,031
	AβR	0,808± 0,025	0,669± 0,029	0,168± 0,033	0,115± 0,015	0,111± 0,013	0,168± 0,028
ISI600 Dev	**S**	0,815± 0,023	0,701± 0,025	0,278± 0,052	0,130± 0,009	0,251± 0,045	0,218± 0,031
	SR	0,828± 0,021	0,713± 0,037	0,405± 0,096	0,413± 0,073	0,358± 0,066	0,304± 0,084
	Aβ	0,809± 0,012	**0,292± 0,015** *	0,248± 0,034	0,190± 0,029	0,206± 0,027	0,207± 0,026
	AβR	0,813± 0,021	**0,630± 0,027** ξ	0,134± 0,014	0,080± 0,030	0,080± 0,017	0,085± 0,026

* : Significant difference versus sham group

ξ : Significant difference versus Aβ group