Increased frontal phase-locking of event-related theta oscillations in Alzheimer patients treated with cholinesterase inhibitors

G.G. Yener a,b,⁎, B. Güntekin c,d, A. Öiniz b, E. Başar c

a Dokuz Eylül University, Faculty of Medicine Departments of Neurology and Neural Sciences, 35340, İzmir, Turkey
b Multidisciplinary Brain Dynamics Research Center, 35340, İzmir, Turkey
c Turkey Istanbul Kültür University, Faculty of Science and Letters, İstanbul, Turkey
d Tübitak Bayg, Ankara, Turkey

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Abstract

This is a pilot study describing event-related oscillations in patients with Alzheimer-type dementia (AD). Theta responses of 22 mild probable AD subjects according to NINCDS-ADRDA criteria (11 non-treated, 11 treated by cholinesterase inhibitors), and 20 healthy elderly controls were analyzed by using the conventional visual oddball paradigm. We aimed to compare theta responses of the three groups in a range between 4–7 Hz at the frontal electrodes. At F3 location, theta responses of healthy subjects were phase locked to stimulation and theta oscillatory responses of non-treated Alzheimer patients showed weaker phase-locking, i.e. average of Z-transformed means of correlation coefficients between single trials was closer to zero. In treated AD patients, phase-locking following target stimulation was two times higher in comparison to the responses of non-treated patients. The results indicate that the phase-locking of theta oscillations at F3 in the treated patients is as strong as the control subjects. The F4 theta responses were not statistically significant between the groups. Our findings imply that the theta responses at F3 location are highly unstable in comparison to F4 in non-treated mild AD patients and cholinergic agents may modulate event-related theta oscillatory activities in the frontal regions.

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1. Introduction

Alzheimer’s disease (AD) is a common form of dementia, constituting over half of cases. With the recent developments in pharmacotherapy of AD, diagnosis and monitoring treatment effects are among the major issues in management of dementia. Within the last decade, the most common therapeutic strategy of AD has been the administration of cholinesterase inhibitors (AChEI).

To date, many signal-processing techniques were utilized in order to reveal pathological changes in spontaneous EEG associated with AD (Jeong, 2004). Theta activity in the spontaneous EEG had been reported to be different in AD than controls (Yener et al., 1996). Furthermore, spontaneous EEG has been suggested as a useful predictive technique in patients with mild cognitive impairment who will later develop AD (Cichecki et al., 2005).

The use of quantitative evaluation of only certain periods of spontaneous EEG poses some limitations in reflecting responses in different frequency bands. Temporal summation of EEG responses after stimulation is different in AD than controls (Polich and Herbst, 2000). The search for the functions of brain oscillations is now an important trend in neuroscience, since oscillatory activity in various frequency bands may reflect different aspects of information processing (Başar, 2004, 1980; Karakas et al., 2000). It is now clear that even the simplest cognitive functions involve large-scale neural networks, most of the results gained after EEG or derived techniques try to describe the related networks (Başar, 1999). Coherence (Gardner, 1992) or phase-locking statistics (Lachaux et al., 2002) are some of the common techniques used to evaluate those relationships.

Event-related theta oscillations have been proposed to be related to the memory processes (Klimesch et al., 1997)
Cholinergic agents have been shown to cause a response increase at a range of 4–15 Hz in the snail brain, (Schütt and Basar, 1992) or to promote firing rates in cats (Eysel et al., 1986) and to increase synchronization of the gamma oscillations in visual cortex after intracortical application (Rodriguez et al., 2004). The question whether cholinergic mechanisms affect or modulate the event-related oscillations in human subjects still remains to be clarified.

A special responsiveness of the frontal lobe in the theta frequency range has been demonstrated previously, in a time prediction task in humans (Başar-Eroğlu et al., 1992) and in a paradigm with regular omitted stimuli in cats (Demiralp et al., 1994). In these studies, the theta responsiveness in frontal lobes was interpreted as an indication of the function of the hippocampo–fronto–parietal system during cognitive processes.

In the present study, we aimed to investigate the phase-locking of visual event-related theta oscillations in frontal locations in two groups of AD and elderly controls. We hypothesized that the non-treated AD would show weaker phase-locking of theta oscillations than both controls and the AD group treated with AChEI.

2. Materials and methods

2.1. Subjects

A prospective open study was conducted. Twenty-five consecutive, community-dwelling patients suffering from dementia according to the DSM IV criteria and also with the diagnosis of probable Alzheimer disease according to the NINCDS-ADRDA criteria (McKhann et al., 1984) were referred to the study. Three of the AD patients were excluded from the final analysis because of excessive motor artifact in their EEG. We analyzed 22 mild probable AD subjects. AD group was divided into two subgroups as the treated and the non-treated. In the treated AD group, eleven subjects (4 males, 7 females) were taking only cholinesterase inhibitors (AChEI) as a psychotropic agent from 3 to 6 months including the titration period (eight subjects were on donepezil 10 mg/d with the initial dose of 5 mg/d that was titrated to 10 mg/d by 4 weeks, and three subjects were on rivastigmine 6–9 mg/d with the initial dose of 3 mg/d, titrated every 4 weeks either to 6 mg/d or to 9 mg/d depending on the tolerance of the drug) and eleven AD patients (4 males, 7 females) not taking any psychotropic medication comprised the non-treated AD group. Both AD groups did not differ from each other regarding Folstein’s Mini-Mental State Examination (MMSE) scores, Reisberg’s Global Deterioration Scale (GDS), gender, education, age, or handedness as shown in Table 1. Disease duration was between one and two years in both of the AD groups. The MMSE scores of all AD subjects ranged between 20 and 24, whereas those of healthy subjects were between 28 and 30 points. All of the AD subjects were in stage 4 according to the GDS. Twenty-two healthy elderly control subjects volunteered for the study, two subjects were excluded for motor artifacts, the remaining 20 control subjects (12 males, 8 females) were not significantly different from both AD groups regarding age, gender, handedness and education (Table 1). All AD subjects underwent a thorough cognitive and complete neurological, neuro-imaging (CT or MRI) and laboratory examination including blood glucose, electrolytes, liver and kidney function tests, full blood count, erythrocyte sedimentation rate, thyroid hormone, vitamin B12, HIV, VDRL. Healthy controls were recruited from various community sources; none of them were relatives of the patients. The study was approved by the local ethics committee. All subjects and relatives gave written informed consent.

2.2. Stimuli and paradigms

Classical P300 oddball paradigm was used in the experiments. The visual stimuli had 10 ms r/f time, 1 s duration and were presented through the monitor. Two types of stimuli were used: the standards and the deviants. The luminance of the standard stimuli was 35 cd/cm² and the deviant stimuli were 20% lower. In all the paradigms, the deviant stimuli were embedded randomly within a series of standard stimuli. The probability of the deviant stimuli was 0.20 and that of standard stimuli 0.80. During the elicitation period of event-related oscillations, all subjects displayed enough accuracy of mental count of target stimuli, being slightly worse in both groups of AD than that of controls.

2.3. Electrophysiological recording

The EEG was recorded from F3, F4, Cz, C3, C4, T3, T4, T5, T6, P3, P4, O1 and O2 locations according to the International
For the recordings an EEG-CAP was used. For the reference, EMG and EOG recordings Ag/AgCl electrodes were used. Linked earlobe electrodes (A1+A2) served as reference. EOG from medial upper and lateral orbital rim of the right eye was also registered. The EEG was amplified by means of a Nihon Kohden EEG-4421 G apparatus with band limits 0.1–100 Hz 24 dB/octave. The EEG was digitized on-line with a sampling rate of 512 Hz and a total recording time of 2000 ms, 1000 ms which served as the pre-stimulus baseline.

2.3.1. Computation of selectively averaged ERPs

Before the averaging procedure, the epochs with artifacts were rejected by an off-line technique. In the off-line procedure, single sweep EOG recordings were visually studied and trials with eye-movement or blink artifacts were rejected. Subject averages and grand averages were calculated for each electrode site and experimental condition. The data was digitally filtered according to determined frequency bands of interest.

In the present study, two approaches were taken in determining the frequency responses of the system: the transient response frequency characteristics (TRFC) method and digital filtering (DF) method.

2.3.2. Digital filtering

Filtering produces visual displays of the time courses of oscillatory components within the frequency limits of the utilized filters. The digital filters are advantageous because they do not produce the phase shifts that are characteristic of electronic filters. The digital filtering was employed in the present study for the digital pass-band filtering of the event-related potentials (ERPs) and thus to demonstrate the event-related oscillations (EROs) in selected frequency-bands (delta: 0.5–3.5 Hz, theta: 4–7 Hz, alpha: 8–13 Hz, beta: 15–30 Hz and gamma: 28–48 Hz) (Başar, 2004).

2.4. Correlation analysis of single sweeps and statistical analysis

Spectral signal analysis constitutes one of the most important and most commonly used analytical tools for the evaluation of neurophysiological signals (Bruns, 2004). Coherence or phase-locking are important coupling measures derived from it.

Correlation analysis was used for the statistical estimation of the covariance of oscillations within single sweeps, within a 350 ms time window (i.e. between +50 and +400 ms), therefore may be considered as a measure for phase-locking. Each single sweep within this time window was presented by a discrete time series of the amplitudes, at, t=1, 2, 3, ..., 179. Correlation coefficients were computed for each pairwise combination of such time series. The obtained correlation coefficients were converted into Fisher’s Z-values \[ Z = \frac{1}{2} \ln \left(1 + \frac{r}{1 - r}\right) \] and then averaged. The arithmetic mean of Fisher’s Z-values was considered to be a measure of similarity for the theta oscillations in the interval analysis. The mean Z-values increase when the oscillations get phase aligned, and close to “0” where sweeps have divergent behavior (Maltseva et al., 2000).

### Table 2

<table>
<thead>
<tr>
<th>Site</th>
<th>Subjects</th>
<th>Pairwise comparisons (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=20)</td>
<td>AD (n=22)</td>
<td>Non-treated vs. Non-treated Controls vs. Non-treated</td>
</tr>
<tr>
<td>Non-treated (n=11)</td>
<td></td>
<td>AD AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.47 (0.37)</td>
</tr>
<tr>
<td>F3 Average of means (SD)</td>
<td>0.40 (0.27)</td>
<td>0.32 (0.15)</td>
</tr>
</tbody>
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Fig. 1. Examples from each group showing single sweeps to the target stimuli elicited by a classical visual oddball paradigm recorded from the scalp electrode of F3. The black and thick line indicates the average of single sweeps, and the grey and thin lines show each single sweep for the subject. A. An elderly healthy control. B. A non-treated Alzheimer subject. C. A treated (cholinesterase inhibitor) Alzheimer subject.
SPSS was used for statistical analysis and the differences between the groups were tested by Kruskal–Wallis and post-hoc LSD tests. Table 2 describes group averages of means of the $Z$-transformed single sweep correlation coefficients in a time window of $+50$ and $+400$ ms at the F3 and F4 locations.

3. Results

We focused our investigations on phase-locking of oscillatory responses in the theta range at the frontal locations and divided probable mild AD subjects into two sub-groups: 1) the non-treated, i.e. those without any psychotropic medication, 2) the treated, i.e. subjects on cholinesterase inhibitors (donepezil or rivastigmine).

3.1. The non-treated AD group

The non-treated AD group showed a weaker phase-locking at the left frontal area (F3) location compared to controls and the treated AD group with lower average mean of $Z$-transformed correlation coefficients (0.238) (Maltseva et al., 2000). In the F4, phase-locking was more pronounced when compared to the F3 location, as observed by higher $Z$-transformed correlation coefficients (Table 2).

3.2. Control group

The mean average of $Z$-transformed correlation coefficients was 0.465 in the F3 electrode indicating that a strong phase-locking existed for the control group. Since in every measurement there were at least 15 trials, this value indicates a stronger phase-locking. At the F3 location, the control group was different than the non-treated AD group, but not from the treated AD group (Table 2).

3.3. The treated AD group

At the F3 location, the treated AD group displayed a phase-locking similar to that of controls. Their average value of mean $Z$-transformed correlation coefficients, 0.525, was much significantly higher than the non-treated AD group. A significant phase-locking was observed in all subjects of the treated AD group both at the F3 and F4 locations (Table 2).

Our results indicated that at the F3 location, the non-treated AD patients had a weaker theta response both than controls and the treated AD groups. This result was related to the less phase-locking in this group (Figs. 1 and 2).

4. Discussion

In the present study, we compared phase-locking of the event-related theta oscillations in untreated and AChEI treated mild AD patients with that of healthy elderly controls. Evaluation of phase-locking of the visual event-related theta oscillations over frontal regions indicates that non-treated AD have lower phase-locking both from controls and AD group treated with AChEI at F3. Treated AD and controls are not significantly different from each other.

Among the neurophysiological methods used, the event-related oscillatory activity in AD had been rather scarcely investigated. The only existing preliminary report on the event-related oscillatory activity in AD has reported that both AD group and controls show event-related synchronization during retrieval period in theta frequency band ($5–7$ Hz), i.e. a frequency range which is commonly considered to be related to working memory, and surprisingly do not differ between the groups, whereas the significant differences have been noted in alpha ($7–17$ Hz) range over frontal, central and left temporal electrodes (Karrasch et al., 2006). The phase-locking of event-related oscillations in AD patients has not been investigated before.

Cortical acetylcholine (Ach) is hypothesized to modulate either the general efficacy of the cortical processing of sensory or associational information or more specifically to mediate the subjects’ abilities to select stimuli and associations for further processing (Sarter et al., 2005). Basal
forebrain is the main source of Ach in the neocortex and Alzheimer patients show depletion in cortical Ach due to degeneration of basal forebrain early in the course of illness. Not only basal forebrain but also glutamatergic and cholinergic mechanisms within prefrontal cortex may regulate Ach release in other parts of cortex such as posterior parietal cortex (Nelson et al., 2005). The ability of prefrontal cortex to regulate transmission in more posterior cortical regions may represent a “top–down” mechanism to control attention (Sarter et al., 2005). For example, thalamocortical fibers are suppressed much less than intracortical connections by acetylcholine, thus possibly enabling the afferent input to have a relative effect in the cortex (Kimura et al., 1999). Therefore, the detrimental performance effects of an ongoing distracter are diminished most likely by increasing the cholinergic processing of sensory inputs (Sarter et al., 2005).

Earlier experimental studies indicate that cholinergic input facilitates induction of long-term potentiation in entorhinal cortex and hippocampus (Yun et al., 2000). Neocortical brain slices had been shown to display oscillatory activity in the 3- to 12-Hz rhythmic slow activity range when perfused with carbachol, a cholinergic agonist, and bicuculline, a g-aminobutyric acid-A (GABAA) antagonist (Lukatch and Maciver, 1997). On the other hand, a close relationship between cholinergic basal forebrain and visual cortex exists. Kainic acid lesions of the basal forebrain reduce both choline acetyltransferase-positive terminals by 90% in visual cortex and the number of neurons that respond to visual stimulation. Local replacement of Ach via microiontophoresis immediately restores the visual response in more than half of the nonresponsive cells (Sato et al., 1987).

In humans, administration of AChEI increases selectivity of neural responses in extrastriate cortices during visual working memory, particularly during encoding as shown with functional MRI (Furey et al., 2000). These agents can restore age-related impairment of functional regional cerebral blood flow (rCBF) response to the vibrotactile stimulation in the normal aged (Tsukada et al., 2000) or improve the latencies of the visual P300 in AD patients (Reeves et al., 1999).

According to new approaches, the EEG consists of the activity of an ensemble of generators producing rhythmic activity in several frequency ranges. These oscillators are active usually in a random way. However, by application of sensory stimulation these generators couple and act together in a coherent way. This synchronization and enhancement of EEG activity gives rise to “evoked” or “induced rhythms”. Evoked potentials representing ensembles of neural population responses were considered as a result of transition from a disordered to an ordered state (Yordanova and Kolev, 1998). The event-related theta oscillations are highly correlated with mechanisms of associative learning and attention as well as retrieval, and frontal theta has a response controlling function (Başar et al., 2001a, b, c). Our findings indicating a weak phase-locked activity in the non-treated AD and a stronger phase-locked activity in the treated AD in theta oscillations over the left frontal area may imply that the left hemisphere may be more prone to be affected first and more readily modulated by cholinergic medication in the early course of the disease. Asymmetric left hemisphere changes were also mentioned in an earlier ERP study showing less synchronization in alpha frequency ranges over the left temporal area (Karrasch et al., 2006). Earlier functional imaging studies showed that after administration of AChEI, clinical responders to treatment selectively display improvements over left prefrontal areas. Potkin et al. (2001) have found prefrontal and hippocampal metabolic increases after rivastigmine. Vennerica et al. (2002) evaluated brain perfusion with rivastigmine treatment and responders showed increased bilateral anterior cingulate and left dorsolateral frontal–parietal perfusion compared with baseline. Nobili et al. (2002) also explored brain perfusion in stable versus nonstable AD patients treated with either donepezil or rivastigmine, and at the follow-up the nonstable group had a frontal defect compared to the stable group. Mega et al. (2005) measuring brain metabolism stated that cognitive responders significantly increased metabolism in the left anterior cingulate after treatment with galantamine. These effects of enhancing attention and executive functions of AChEI may be explained by monosynaptic connections of anterior cingulate with dorsolateral prefrontal association cortices (Mesulam, 1985). Since the majority of our treated AD subjects remained stable or improved over the 3–6 months period (same or higher MMSE score than baseline) after the treatment, we believe that stronger phase-locking observed in left frontal area may be in line with earlier functional imaging studies mentioned above. However, even in the presence of a cognitive response to the currently available AChEI, the improvements in daily activities are far from the expected, implying the fact that not only cholinergic, but many other neurotransmitter systems are involved in AD.

In earlier AD, an amnestic syndrome is essential and the corresponding neuropathological changes are seen in medial temporal lobes and biparietal areas. Therefore, neurophysiologic correlates are expected over these areas. Even though structurally frontal lobes seem to be preserved at early stages, the strong functional connections arising from the affected limbic or heteromodal areas to the frontal areas may be the cause of changes in the function of the frontal lobes, as shown by transgenic animal models of AD (Delatour et al., 2004) or PET-based functional connectivity mapping (Grady et al., 2001), and EEG coherence recordings (Leuchter et al., 1992). A recent study on theta oscillations of schizophrenia patients indicated reduced late theta responses from all locations, suggesting that theta oscillations are involved in mediating frontal lobe activity and functions related to enhanced executive control (Schmiedt et al., 2005b). Furthermore, the changes in the long distance fronto–parietal interactions during visual working memory retention and mental imagery may result in the oscillatory changes of theta and alpha frequency ranges (von Stein and Sarnthein, 2000).

Our results indicate that at F3 location the non-treated AD patients have a weaker theta response. This may be the result of
less phase-locking, in contrast to the treated patients who show stronger phase-locking. Therefore the cholinergic agents may improve the phase-locking of responses or responsiveness of the brain to cognitive targets.

The difference in phase-locking of theta frequency band between treated and non-treated AD patients in the left frontal area is a novel observation. If further clinical studies confirm, it can be a tool for the reflection of deficits seen in AD or the response to the treatment. Also missing theta response may further demonstrate the role of working memory in the generation of theta oscillations in the non-treated AD. The theta oscillations in the treated AD similar to controls may indicate that the cholinergic pharmacotherapy help synchronize of theta oscillations. Yet it is not conclusive whether this difference in phase-locking of theta oscillations between treated and non-treated patients is completely due to cholinergic effects. Studying event-related oscillations may open a new venue to detect pharmacologic influences. In the next step, we intend to compare results of non-treated patients prior to and after medication. For the time being, the differentiation or effect from the cholinergic drugs seems to be highly meaningful.

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References


