Clinical Neurophysiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

A 15-day course of donepezil modulates spectral EEG dynamics related to target auditory stimuli in young, healthy adult volunteers

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ARTICLE INFO

Article history:
Accepted 20 November 2015
Available online xxxx

Keywords:
Donepezil
EEG markers
Event-related potential
Inter-trial coherence
Event-related spectral perturbation

HIGHLIGHTS

- Time–frequency analyses were used to identify dynamic electroencephalographic markers of donepezil's effect in healthy young adults.
- Inter-trial coherence and event-related spectral perturbation analyses can detect subtle changes related to donepezil's effects.
- Electroencephalography may be a valuable tool for predicting the efficacy of drug candidates prior to Phase II/III clinical trials in Alzheimer's disease.

ABSTRACT

Objective: To identify possible electroencephalographic (EEG) markers of donepezil's effect on cortical activity in young, healthy adult volunteers at the group level.
Methods: Thirty subjects were administered a daily dose of either 5 mg donepezil or placebo for 15 days in a double-blind, randomized, cross-over trial. The electroencephalogram during an auditory oddball paradigm was recorded from 58 scalp electrodes. Current source density (CSD) transformations were applied to EEG epochs. The event-related potential (ERP), inter-trial coherence (ITC: the phase consistency of the EEG spectrum) and event-related spectral perturbation (ERSP: the EEG power spectrum relative to the baseline) were calculated for the target (oddball) stimuli.
Results: The donepezil and placebo conditions differed in terms of the changes in delta/theta/alpha/beta ITC and ERSP in various regions of the scalp (especially the frontal electrodes) but not in terms of latency and amplitude of the P300-ERP component.

Abbreviations: AD, Alzheimer's disease; EEG, electroencephalographic; AChEI, acetylcholinesterase inhibitor; ERP, event-related potential; ITC, inter-trial coherence; ERSP, event-related spectral perturbation; CSD, current source density; ERS, event-related-synchronization; ERD, event-related-desynchronization; PCA, principal component analysis.

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http://dx.doi.org/10.1016/j.clinph.2015.11.018

Conclusion: Our results suggest that ITC and ERSP analyses can provide EEG markers of donepezil's effects in young, healthy, adult volunteers at a group level.
Significance: Novel EEG markers could be useful to assess the therapeutic potential of drug candidates in Alzheimer's disease in healthy volunteers prior to the initiation of Phase II/III clinical studies in patients.

1. Introduction

The currently available pharmacological treatments for Alzheimer's disease (AD) lack long-term effectiveness (Clegg et al., 2001; Hansen et al., 2008; Persson et al., 2009). Furthermore, the cognitive tests generally used to rapidly assess the clinical benefit of symptomatic drugs in AD patients (such as the Mini Mental State Examination (Simard, 1998) and the cognitive subscale of the Alzheimer Disease Assessment Scale (Schafer et al., 2011)) lack the sensitivity required to reveal subtle changes in cognitive performance. To circumvent these obstacles, there is a need for innovative ways of evaluating the efficacy of AD drug candidates in clinical trials. This might involve the incorporation of biomarkers in early-phase clinical studies (Deguil et al., 2013). Innovative strategies would save time and money and would reduce the number of participants needed to demonstrate the therapeutic benefit of a new medication.

In this context, it has been hypothesized that the addition of electrophysiological measurements of cortical activity to the current battery of cognitive tests would provide new insight into drug-related changes in neural functioning (Babiloni et al., 2006; Balsters et al., 2011; Reches et al., 2013). Given the millisecond resolution of electroencephalography (Gosseries et al., 2008), this technique may reveal subtle dynamic changes in the brain activity underlying cognitive functions (Klimesch, 1999; Güntekin and Başar, 2010; Klimesch, 2012; Peng et al., 2012). Hence, electroencephalography might constitute an additional tool for predicting the efficacy of AD drug candidates in Phase I clinical trials (i.e. those performed with healthy volunteers). Nevertheless, the value of this type of tool must first be confirmed with currently marketed AD drugs.

Donepezil is a potent, non-competitive, reversible, clinically effective acetylcholinesterase inhibitor (AChEI) that is primarily used to reduce cognitive impairments in mild-to-moderate AD (Seltzer et al., 2004). Use of this medication is associated with slower deterioration of electroencephalographic (EEG) activity in AD in the resting state (Babiloni et al., 2006) and during cognitive tasks (Katada et al., 2003; Chang et al., 2014). In particular, analyses of event-related potentials (ERPs) have shown that donepezil can significantly reduce the latency of the P300 component in AD patients (Reeves et al., 1999; Katada et al., 2003; Chang et al., 2014). However, these donepezil-induced changes in P300 are not specific to AD and are also observed in vascular dementia (Paci et al., 2006). Dynamic spectral analyses of theta-event-related oscillations during a visual oddball paradigm have shown that donepezil might modulate phase-locking in the frontal regions in patients with mild AD (Yener et al., 2007). In contrast, another study by the same researchers showed that untreated and AChEI-treated AD patients displayed similar delta-event-related oscillations (with both patient groups differing significantly from healthy controls) (Yener et al., 2012). Two studies performed by Yener et al. (2007, 2012) suggest that (i) theta-event related oscillations are EEG markers of cholinergic modulation by AChEIs in AD patients, and (ii) delta-event-related oscillations are more sensitive to the cognitive decline caused by AD than the cholinergic modulation caused by AChEIs.

In studies of healthy subjects, donepezil has been found to influence cognitive processes in general and attentional mechanisms, executive functions and memory in particular (Gron et al., 2005; Zaninotto et al., 2009). Resting-state EEG markers of donepezil's action have been identified in healthy elderly participants, as attested to by an increase in delta power, a decrease in alpha power, an increase in hippocampus activity and a decreased activity of the default mode network (Balsters et al., 2011). However, little is known about donepezil's effect on dynamic EEG patterns in healthy volunteers. To date, only one EEG study has investigated the dynamic effect of donepezil during a delayed recognition paradigm; the drug appeared to modulate a frontal-posterior theta-alpha attentional network whose activation was positively correlated with working memory performances. In contrast, there was no difference between the placebo and donepezil groups in terms of cognitive performance during the task (Reches et al., 2013). The investigation of dynamic electrophysiological patterns in healthy subjects might reflect subtle drug-related changes in neuronal processes directly related to the performance of a cognitive task.

The primary objective of the present study was therefore to identify possible dynamic EEG markers of donepezil's effect in young, healthy, adult volunteers. In general, the brain's electrical activity changes with age. Recordings of the resting EEG activity indicate that there is a gradual, age-related change in the power spectral profile, with (i) a pronounced decrease in power in the alpha band (8–13 Hz), (ii) an overall slowdown of the background EEG activity and (iii) an increase in delta (2–4 Hz) and theta (4–7 Hz) slow activity (Klimesch, 1999). We therefore chose to track these markers in healthy young adults, in order to reduce the inter-individual variability in age-related cognitive function. Furthermore, we wanted to ensure that any observed changes were attributable to the effects of the drugs and not to age-related electrophysiological changes. We employed an auditory oddball paradigm that is conventionally used to study the dynamics of neural processes involved in cognitive function (Sutton et al., 1965; Katayama and Polich, 1999; Spencer and Polich, 1999; Sutoh et al., 2000; Yordanova et al., 2001; Höller et al., 2013). We focused on two advanced mathematical parameters in EEG analysis: inter-trial coherence (ITC, reflecting the phase consistency of the EEG spectrum) and event-related spectral perturbation (ERSP, reflecting the EEG power spectrum with respect to the baseline). ITC and ERSP are sensitive enough to detect subtle changes in human cortical activity during an oddball paradigm (Ko et al., 2012; Güntekin et al., 2013). Indeed, some changes in stimulus-induced ongoing EEG activity contain important information on cognitive processes (Makeig et al., 2004) that cannot be extracted by averaging the EEG voltage–time data (Pfurtscheller and Lopes da Silva, 1999). Thus, we hypothesized that ERP, ITC and ERSP analyses of EEG signals during an auditory oddball paradigm could provide dynamic markers of the effect of donepezil. Furthermore, we sought to reduce the effect of brain volume conduction (and thus

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improve the detection of any subtle differences) by calculating the current source densities (CSDs) of the EEG data; this method is known to improve the spatial distribution of scalp EEG signals (Babiloni et al., 2001; Kayser and Tenke, 2006).

2. Methods

2.1. Participants

Thirty male, right-handed, young, healthy adults aged between 19 and 30 (mean ± SD age: 24.6 ± 3.1) were included in the study. None of the participants had known neurological or psychiatric disorders, smoked or was taking chronic treatment or psychoactive drugs. All participants gave their written, informed consent to participation. The study was approved by both the local independent ethics committee and the French drug safety authorities (*Agence Nationale de Sécurité du Médicament et des Produits de Santé*). The study also complied with good clinical practice guidelines, the Declaration of Helsinki, and local legislation. It was registered under the European policy number EudraCT 2010-023989-51 and the Clinicaltrial.gov identifier NCT01487395.

The study had a double-blind, randomized, cross-over design (Fig. 1). Each participant was randomly allocated to receive either (i) donepezil in the first session and placebo in the second session or (ii) with placebo in the first session and donepezil in the second session. Donepezil (5 mg/day) or placebo was administrated orally in the morning over a 15-day period. The two sessions were separated by a one-month wash-out period (given the 70-h half-life of donepezil (Noetzli and Eap, 2013)). EEG recording was performed at the end of each session.

2.2. The experimental task

The auditory oddball paradigm included 200 frequent auditory tones (80%) with a frequency of 500 Hz and 50 intermingled, target auditory tones (20%) with a frequency of 1000 Hz. Each tone was presented for 50 ms, with a fixed inter-stimulus interval of 1550 ms. Participants were comfortably seated, and auditory tones were displayed binaurally in a random manner. Participants were instructed to press a joystick button with the index of their dominant (i.e. right) hand immediately after the onset of a target tone. The EEG session lasted approximately seven minutes.

2.3. EEG recordings

The electroencephalogram was recorded from 58 electrodes placed according to the 10/10 international standard system (Oostenveld and Praamstra, 2001) with a linked mastoid reference (M1–M2). The positioning of the electrode cap was guided by fiducial points: nasion, inion and preauricular points. Electrode impedances were kept below 10 kΩ. We used Brain Vision Recorder software (version 1.20.0701, Brain Products GmbH, Munich, Germany) for data acquisition. The EEG was recorded with an analog band-pass filter set to 0.1 to 100 Hz and digitized with a sampling rate of 500 Hz. Horizontal and vertical eye movements were recorded via four reference electrodes (bipolar montage) for the further correction of EEG recordings.

2.4. Pre-processing

Brain Vision Analyzer software (version 2.0.4, Brain Products GmbH) was used to pre-process the EEG recordings. First, EEG data were filtered with a 50 Hz notch filter to remove residual noise. Ocular artifacts were then semi-automatically detected and corrected according to Gratton and Coles' method (Gratton et al., 1983). The EEG signals were then segmented from −450 ms (relative to the stimulus onset) to 1300 ms. Epochs were classified as "frequent" and "target", according to the type of stimulus presented. A non-automated, visual inspection was used to reject epochs containing EEG artifacts and those not related to the correct behavioral response (i.e. false positives for the frequent stimuli and omissions for the target stimuli). On this basis, six participants were excluded because of the low number of valid epochs (less than 25) for the target stimulus. Hence, analyses were performed for 24 of the 30 participants. All additional analyses on EEG epochs were performed with MATLAB® (version R2010a, MathWorks, Natick, MA, USA).

2.5. Processing

2.5.1. CSD transformations

CSD transformations are known to improve the spatial resolution of scalp EEG signals; they yield sharper scalp topographies (Tenke et al., 1998). Furthermore, the CSD-EEG measurements are independent of the recording reference (Kayser et al., 2010, 2014). This approach also reduces the impact of brain volume conduction on the results (Hjorth, 1975; Babiloni et al., 2001; Kayser and Tenke, 2006). In the present study, the CSD was calculated by using the spherical spline Laplacian method developed by Perrin et al. (1989), with the same suggested parameter values (50 iterations; $m = 4$; $\lambda = 10^{-5}$). These estimations were performed at each electrode in a unit sphere (radius $r = 1.0$). Lastly, the values were scaled to Laplacian units ($\mu V/cm^2$) on the basis of a more realistic head radius (10 cm). CSD transformations were applied to the

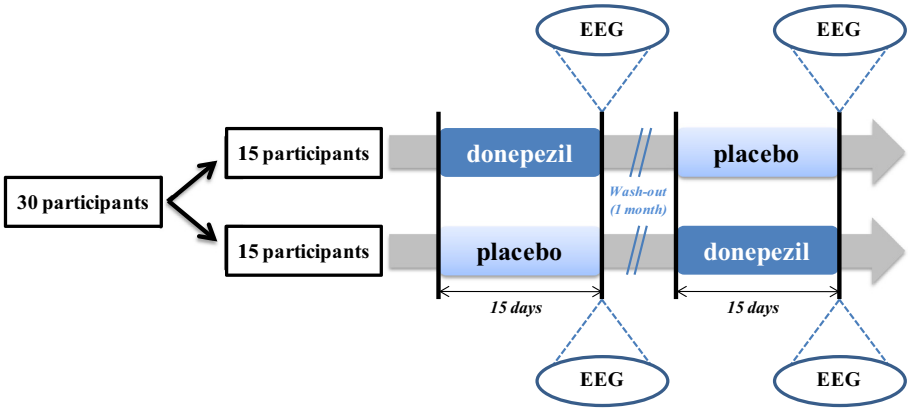

Fig. 1. The experimental design.

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single-trial EEG epochs associated with the target stimuli on one hand and the frequent stimuli on the other.

2.5.2. ERPs

For the target stimulus, CSD-EEG epochs were averaged in order to obtain ERPs. Baseline corrections were applied between −150 ms and −50 ms (relative to the stimulus onset). CSD-P300 was identified as the largest positive deflection between 250 ms and 450 ms. For each of the two drug conditions (i.e. donepezil and placebo), the CSD-P300 peak amplitude and peak latency (in the 250–450 ms interval) were calculated for the target stimulus at Fz, Cz, CPz, Pz, POz and Oz locations.

2.5.3. ITC and ERSP

Time–frequency variations of the CSD-EEG epochs induced by the auditory oddball task were assessed in ITC and ERSP analyses (Delorme and Makeig, 2004).

2.5.3.1. ITC. ITC is a measure of the degree of phase synchronization between EEG activities across trials at a given time and frequency, in response to a stimulus. It quantifies the extent to which the post-stimulus phase value remains constant from one trial to another, and thus reflects stable dynamic neural processing of each stimulus (Tallon-Baudry et al., 1996; Makeig et al., 2004).

Mathematically, ITC is calculated as follows:

$$\mathrm{ITC}(f,t) = \frac{1}{n}\sum_{k=1}^{n}\frac{F_k(f,t)}{|F_k(f,t)|}$$

where n is the number of trials, and $F_k(f,t)$ is the spectral estimate of the kth trial at frequency f and time t.

ITC ranges from 0 (random phase distribution across trials) to 1 (perfect EEG phase reproducibility across trials).

2.5.3.2. ERSP. The ERSP is a measure of the average dynamic changes in the amplitude of EEG spectra across trials at a given frequency and time. It provides a normalized measure of EEG power spectrum variation in response to stimulation with respect to the baseline power spectrum (i.e. prior to the stimulus). In other words, ERSP enables one to track event-related synchronization (ERS) and event-related desynchronization (ERD) in the EEG power spectrum. ERD corresponds to amplitude attenuation or the blocking of rhythmic components of the EEG in certain event-related frequency bands. It reflects a state of cortical activation (Steriade and Llinás, 1988). Conversely, ERS corresponds to an increase in the amplitude of the event-related rhythmic components of the EEG and is associated with a state of cortical inactivation (Pfurtscheller, 1992).

Mathematically, the ERSP is calculated as follows:

$$\mathrm{ERSP}(f,t) = 20\log\frac{\sum_{k=1}^{n}|F_k(f,t)|}{\sum_{t\epsilon\mathrm{baseline}}\sum_{k=1}^{n}|F_k(f,t)|}$$

where n is the number of trials and $F_k(f,t)$ is the spectral estimate of the kth trial at frequency f and time t.

2.5.4. Time–frequency analyses

The ITC and ERSP parameters were calculated separately for the target stimulus and the frequent stimulus at 29 spectral frequencies (from 2 to 14 Hz in 1 Hz increments, and from 15 to 45 Hz in 2 Hz increments) and 150 time-points (-150 ms to 1300 ms) for each subject, condition (donepezil and placebo), and recording site. Thus, frequency-by-time (29-by-150) ITC and ERSP matrices were obtained. ERSP and ITC were calculated with Morlet wavelet decomposition (with a zero-padding ratio of 4 and 150 time points, 2.5 cycles at the lowest frequency band and 22.5 cycles at the highest frequency band). Next, for ERSP analyses, a baseline reference was taken between −150 ms and −50 ms (relative to the stimulus onset) in order to yield normalized measures. ITC and ERSP analyses were performed with MATLAB® software (version R2010a, MathWorks, Natick, MA, USA) using (i) equations developed in house and (ii) some of the core functions provided in the EEGLAB toolbox (version 12.0.0.0b; Delorme and Makeig, 2004).

Evoked activities are time-locked and phase-locked with respect to the stimulus onset; in contrast, induced activities are time-locked but not phase-locked (Klimesch, 1999; Pfurtscheller and Lopes da Silva, 1999; Tallon-Baudry and Bertrand, 1999). The ITC analysis enabled us to track the phase-locking values across trials related to the target stimulus. In other words, high values of ITC reflect ERP in the time–frequency domain. Furthermore, the ERSP analysis enabled us to detect ERS and ERD in the EEG power spectrum in response to the target stimulus. Therefore, ITC and ERSP results are complementary. At a given time and frequency, the presence of both ERSP and ITC activities is indicative of evoked activity, whereas ERSP activity with an ITC close to 0 reflects an induced activity. This is why we chose not remove the ERP from the EEG segment, i.e. so that we could analyze evoked and induced activities in the time–frequency domain.

2.6. Statistical analysis

2.6.1. Behavioral data and CSD-ERPs

Student's paired t-test was used to study the effect of substance (donepezil vs. placebo) on behavioral data from the oddball task (the response time and numbers of hits, false positives and omissions).

The latency and amplitude of the CSD-P300 component related to the target stimulus were compared in both conditions and at electrode midlines. A repeated-measures analyses of variance (ANOVA) was applied, with the treatment type (donepezil and placebo) as a between-group factor and the electrode location (Fz, Cz, CPz, Pz, POz and Oz) as a within-group factor. Greenhouse-Geisser correction was performed when the assumption of sphericity was violated. The threshold for statistical significance was set to $p < 0.05$.

2.6.2. Time–frequency results

In order to reduce the size of ERSP matrix, a principal component analysis (PCA) (Bernat et al., 2005) was applied for each type of stimulus (i.e. the target stimulus and the frequent stimulus). The frequency-by-time (29-by-150) matrix was first reduced to a 29-by-20 matrix (from −150 to 700 ms in 50 ms increments and from 700 to 1300 ms in 100 ms increments) and then reorganized into a single vector by concatenating the time vectors for each frequency. The time–frequency PCA was computed using 580 variables (29 frequencies × 20 time epochs) and 2784 observations arising from 24 participants, 2 conditions (donepezil and placebo) and 58 electrode sites. These data (i.e. 580 variables for 2784 observations) were factorized using the covariance matrix and then modified by unscaled Varimax rotation (Kayser and Tenke, 2003). The eigenfunctions corresponding to the highest eigenvalues in the PCA (i.e. those accounting for more 5% of the total variance) were selected for representation in ERSP topographic maps. For each component, the CSD factor scores were pooled across all 24 participants for each condition and each electrode. Next, these values were used to display topographies in which the sign of the factor scores corresponded directly to ERS or ERD. The CSD factor scores associated with the donepezil and placebo conditions were compared by using Student's paired t-test. The threshold for statistical significance was set to $p < 0.05$.

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3. Results

3.1. Behavioral data

The behavioral data from the oddball task are summarized in Table 1. The donepezil and placebo conditions did not differ significantly in terms of the mean response time or the numbers of hits, false positives and omissions ($p > 0.05$).

3.2. Averaged CSD-ERPs

Fig. 2 shows the grand average CSD-ERPs at 58 electrodes for the target stimulus under both conditions (donepezil and placebo). Along the central axis, the CSD-ERP waveforms were similar under the two conditions. In agreement with the results of previous studies of auditory oddball paradigms (Tenke et al., 2008; Kayser et al., 2014), the CSD-P300 waveform was prominent at parietal sites. Table 2 shows the latency and peak amplitude of the CSD-P300 produced at scalp electrodes located on the midline (Fz, Cz, CPz, Pz, POz and Oz) for the target stimulus in the donepezil and placebo conditions. For the latency, an ANOVA did not reveal a significant overall effect of the type of treatment ($F_{(1,46)} = 0.81$, $p = 0.37$) or the electrode location ($F_{(5,46)} = 1.41$, $p = 0.23$). No effect of a treatment × electrode interaction was observed.

For the amplitude, an ANOVA revealed a main effect of electrode location ($F_{(5,46)} = 25.09$, $p < 0.05$). The amplitude increased according to an anteroposterior gradient (Fz < Cz < Pz). However, no significant overall effect was observed for the type of treatment ($F_{(1,46)} = 1.01$, $p = 0.32$). Again, no effect of a treatment × electrode interaction was observed.

3.3. ITC- and ERSP-target topographic maps

Time–frequency values of the ITC and ERSP were displayed as scalp topographies maps for both the donepezil and placebo conditions (Figs. 3 and 4). Each map represents the average ITC and ERSP values from 150 ms before the target stimulus to 1300 ms after (in 20 time epochs). In order to present the results more clearly, we divided the frequency axis into the conventional EEG frequency bands: delta (2–4 Hz), theta (5–7 Hz), alpha (8–14 Hz), beta (15–30 Hz) and gamma (31–45 Hz).

At about 100 ms (and in all frequency bands), our ITC analysis revealed two synchronized foci at the left and right frontotemporal electrodes; these features appeared to be stronger in the donepezil condition (see the green box in Fig. 3). At about 300 ms, phase synchronizations (in delta and theta bands) at the parietal electrodes appeared to be similar under both donepezil and placebo conditions (see the blue box in Fig. 3). For all the other time-epochs and for all the frequency bands, ITC was close to 0 under both conditions (see the red box in Fig. 3).

In the ERSP analysis, ERS appeared to be stronger in the donepezil condition at around 100 ms (in all frequency bands) at the left and right frontotemporal electrodes (see the green box in Fig. 4) and at around 300 ms (in delta and theta bands) at the frontal electrodes (see the blue box in Fig. 4). A greater alpha/beta ERS at frontal electrodes was observed at about 400 ms in the donepezil condition (see the orange box in Fig. 4). Furthermore, an alpha ERD was observed (at around 400 ms) at the centroparietal electrodes in both conditions (see the orange box in Fig. 4). Similarly, delta/theta ERS from 450 ms to 1300 ms was weaker at frontal electrodes in the donepezil condition (see the gray box in Fig. 4). At about 900 ms, beta ERS at the frontal, parietal and occipital electrodes appeared to be weaker in the donepezil condition (see the purple box in Fig. 4). However, beta ERD at temporoparietal electrodes appeared to be stronger in the donepezil condition (see the purple box in Fig. 4).

Statistical analyses of the above-mentioned topographies would have lacked power because of the large number of variables involved (58 electrodes and 20 time-epochs). We performed a PCA of the CSD-ERSP-target data (using the method previously implemented by Kayser et al. (2014)), in order to focus on relevant information contained in the scalp topography maps.

Scalp ITC (Supplementary Fig. S1) and ERSP (Supplementary Fig. S2) topographies for the frequent stimulus are shown as Supplementary material.

3.4. PCA for determining the most relevant CSD-ERSP-target localizations

Fig. 5 shows the ERSP matrices of factor loadings (Fig. 5A) and associated factor score topographies for the first five CSD factors (i.e. PCAs 1, 2, 3, 4, and 5, accounting for 58.67% of the total variance after rotation). It should be noted that the fifth factor loading accounted for less than 5% of the total variance (i.e. 4.3%). However, we chose to retain this factor because it reflected relevant neurophysiologic mechanisms.

For each selected PCA component (and for each electrode), the factor score topographies associated with the donepezil and placebo conditions (Fig. 5B) were compared in a Student's paired *t*-test (Fig. 5C). Statistically significant values are indicated by a red dot on a topographic map for each PCA component. Fig. 5C shows the results of the statistical comparison of the ERSP topographic maps (for the target stimulus) generated under donepezil vs. placebo conditions.

In general, there was a reasonable degree of agreement between the CSD-ERSP-target activities observed in the descriptive analysis (see Section 3.3 and the various colored boxes in Fig. 4) and those identified by the PCA (Fig. 5). PCA 1 (Fig. 5B) may be associated with the purple box in Fig. 4. In fact, PCA 2 may be related to the orange box, with PCA 3 related to the gray box, PCA 4 related to the green box and PCA 5 related to the blue box.

For the sake of clarity, we have chosen to describe these results in the conventional EEG frequency bands.

3.4.1. The delta (2–4 Hz) and theta (5–7 Hz) bands

At about 300 ms after the onset of the target stimulus, delta/theta ERS was observed at frontocentroparietal electrodes under both conditions (see PCA 5 in Fig. 5B). In the donepezil condition, this delta/theta ERS was weaker at the left parietal electrodes (see PCA 5 in Fig. 5C) ($p < 0.05$). Later on (at about 700 ms), delta/theta ERSs appeared at both the frontal and occipital electrodes and were weaker in the donepezil condition – especially at the frontal electrodes (see PCA 3 in Fig. 5B and C) ($p < 0.05$).

As observed in the Section 3.3, our descriptive ITC analysis revealed delta/theta ITC at about 300 ms at the parietal electrodes (see the box blue in Fig. 3). However, no delta/theta ITC activity was observed at about 700 ms (see the red box in Fig. 3). This suggests that PCA 5 and PCA 3 are related to evoked and induced activities, respectively.

Table 1
Mean response time and performance rates for the target stimulus in the donepezil and placebo conditions. The data are expressed as the mean and standard deviation (SD). Paired *t*-tests were used in the statistical analysis ($n = 24$ participants). NS: non-significant to $p \leqslant 0.05$.

	donepezil		placebo		*p* value
	Mean	SD	Mean	SD	
Response time (ms)	339.42	71.77	328.81	50.24	0.45 (*NS*)
Hit targets (%)	99.75	0.89	99.75	0.66	0.98 (*NS*)
False positives (%)	0.18	0.31	0.07	0.14	0.10 (*NS*)
Omissions (%)	0.04	0.21	0.13	0.34	0.33 (*NS*)

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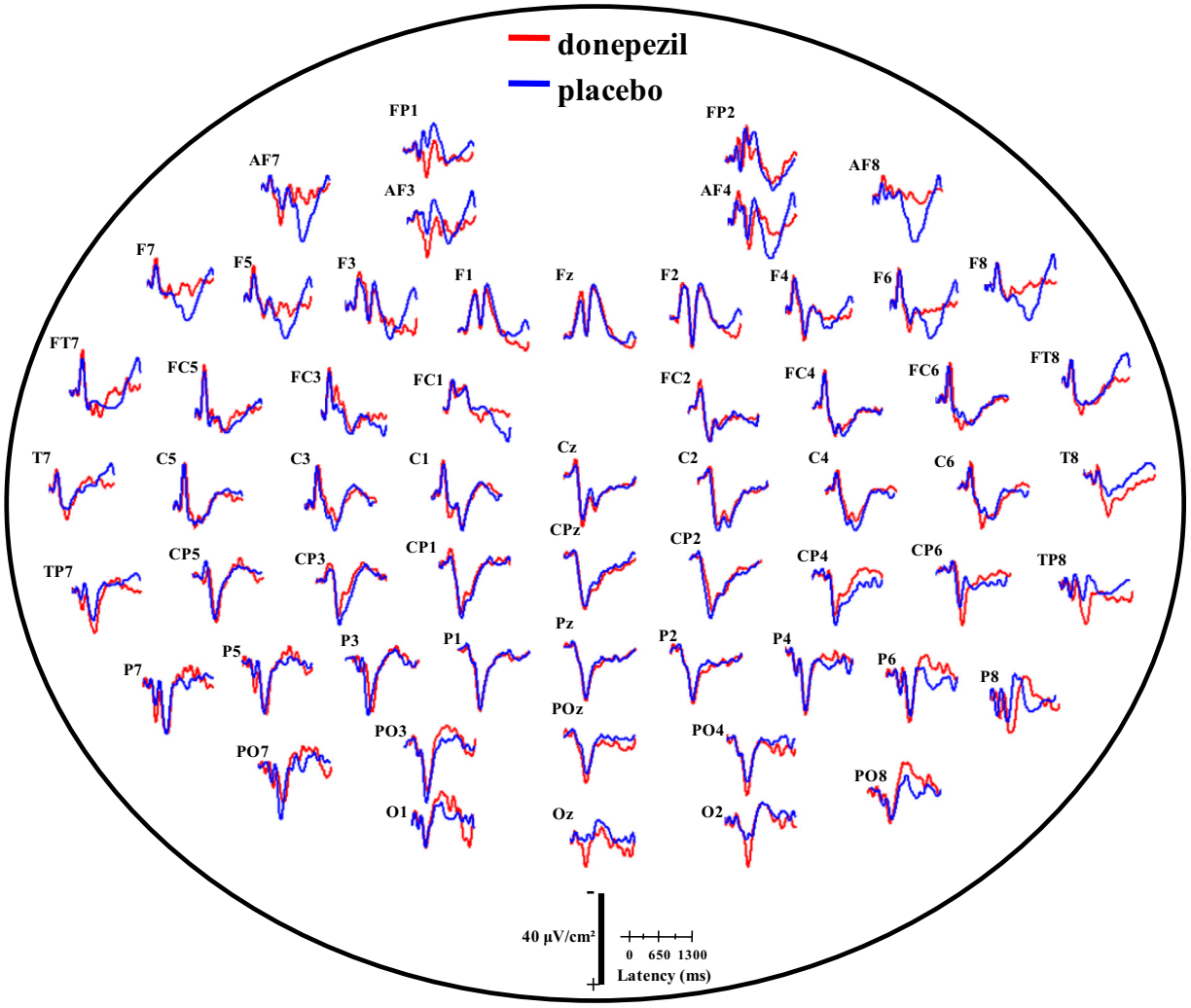

Fig. 2. Grand average surface Laplacian (CSD) waveform (−150 to 1300 ms, with −150 to −50 ms as the pre-stimulus baseline) for the target stimulus under the donepezil and placebo conditions (n = 24 participants).

Table 2
Characteritsics of the latency and amplitude of the CSD-P300 component in the donepezil and placebo conditions for the target stimulus (250–450 ms). The data are expressed as the mean and standard deviation (SD) of the grand average for electrodes on the central axis (Fz, Cz, CPz, Pz, POz and Oz); n = 24 participants.

Electrode	Condition	Mean (SD) peak latency (ms)	Mean (SD) peak amplitude (µV/cm²)
Fz	donepezil	322.75 (50.25)	7.89 (18.03)
	placebo	311.92 (54.02)	9.77 (12.67)
Cz	donepezil	295.75 (65.49)	27.81 (15.57)
	placebo	309.50 (76.31)	24.63 (17.74)
CPz	donepezil	296.58 (54.57)	32.33 (11.88)
	placebo	309.58 (59.29)	29.73 (12.89)
Pz	donepezil	314.42 (43.19)	36.21 (17.01)
	placebo	321.25 (44.09)	33.30 (11.90)
POz	donepezil	313.58 (34.44)	33.54 (20.87)
	placebo	329.08 (50.89)	29.72 (22.08)
Oz	donepezil	345.75 (64.90)	18.30 (14.53)
	placebo	347.75 (70.74)	12.36 (14.26)

3.4.2. The alpha band (8–14 Hz)

At about 100 ms, an alpha ERS was observed at the left and right temporal electrodes under the donepezil and placebo conditions (see PCA 4 in Fig. 5B). The perturbation was greater at a left temporal electrode in the donepezil condition (see PCA 4 in Fig. 5C) ($p < 0.05$). Moreover, this alpha ERS was associated with a high ITC value (see the green box in Fig. 3). Therefore, PCA 4 (Fig. 5B) might be associated with an evoked activity. Furthermore, an alpha/beta-ERD was observed at the centroparietal electrode; it was most prominent on the left side at about 400 ms (i.e. just after the motor response on the contralateral side; see PCA 2 in Fig. 5B). Although this ERD appeared to be more intense in the placebo condition, the inter-condition difference was not statistically significant (see PCA 2 in Fig. 5C). This alpha ERD was not associated with a high ITC value and might therefore reflect induced activity (see the red box in Fig. 3).

3.4.3. The beta band (15–30 Hz)

At about 400 ms, a beta ERS at frontal electrodes was observed under both conditions (see PCA 2 in Fig. 5B) but was greater in the donepezil condition (see PCA 2 in Fig. 5C) ($p < 0.05$). At about 900 ms (see PCA 1 in Fig. 5B), ERSP analyses showed that in the donepezil condition, there was weaker beta ERS at frontal and parieto-occipital electrodes and stronger beta ERD at temporal-parietal electrodes (see PCA 1 in Fig. 5C) ($p < 0.05$). These activities (i.e. PCAs 2 and 1) were not associated with noticeable ITC (see the red box in Fig. 3) and may therefore be attributable to induced activities.

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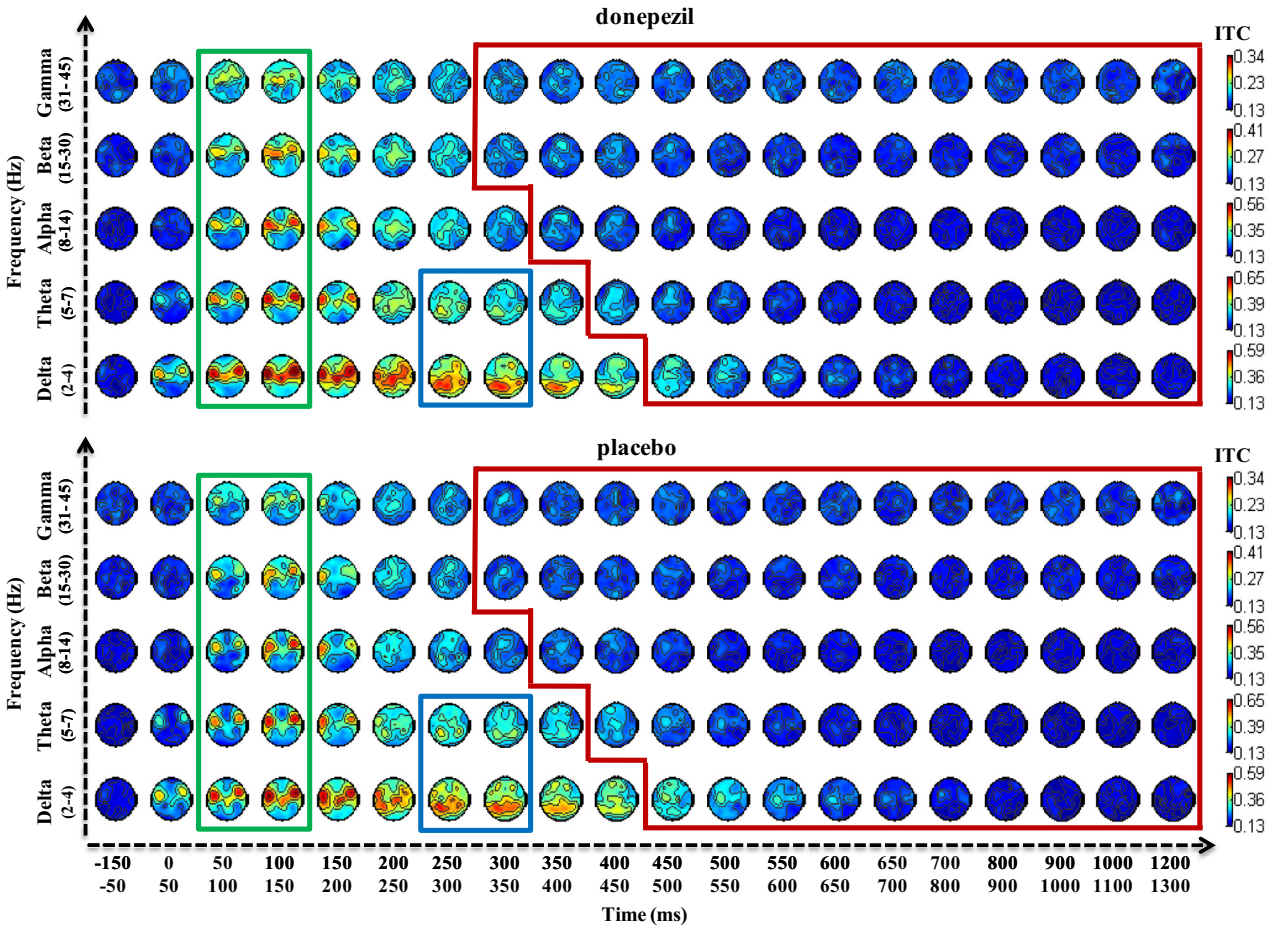

Fig. 3. Scalp ITC topographies for the target stimulus. Each frequency band has its own color scale, which is the same in the donepezil and placebo conditions. Time windows (in milliseconds, ms) are shown on the *x*-axis and frequency bands are shown on the *y*-axis. ITC ranges from 0 (in blue, representing a random phase distribution across trials) to 1 (in red, representing perfect EEG phase reproducibility across trials).

3.4.4. The gamma band (31–45 Hz)

No gamma band components emerged from the PCA (Fig. 5A).

The PCA used to determine the most relevant CSD-ERSP localizations for the frequent stimulus is shown as Supplementary material (Supplementary Fig. S3).

4. Discussion

The present study's primary objective was to identify possible sensitive, specific EEG markers of cholinergic modulation in donepezil-treated, young, healthy, adult volunteers. Our analysis of changes in spectral EEG variables induced by the target stimulus (i.e. CSD-ERSP and CSD-ITC) revealed differences between the donepezil and placebo conditions and thus were suggestive of differences in functional cortical networks. However, analyses of the target CSD-P300 ERP's latency and amplitude failed to show any significant differences between the donepezil and placebo conditions. One previous study has shown that acute (one-month) donepezil administration was associated with a shorter P300 latency during an auditory oddball paradigm in AD patients (Reeves et al., 1999). In turn, a shorter P300 latency is known to be related to improvements in cognitive functions such as short- and long-term memory, attention and orientation in AD (Chang et al., 2014). In the present study, there was no significant difference in behavioral responses when comparing the donepezil and placebo conditions. It is likely that analysis of the P300 component was not sensitive enough to reveal differences in young, healthy, adult subjects. ERP analysis provides an overall representation of EEG activity by including all frequency bands. Time–frequency-based methods decompose this activity and can therefore provide a detailed representation of the EEG-ERP signals (Mazaheri and Picton, 2005). Our ERSP and ITC analyses enabled us to distinguish between evoked activities and induced activities.

4.1. Evoked activities

4.1.1. The delta and theta bands

A delta/theta ERS was observed at frontocentroparietal electrodes under both conditions (at about 300 ms) for the target stimulus. On left parietal electrodes, the delta/theta ERS was weaker in the donepezil condition. An EEG study of a visual oddball paradigm had already shown that during target detection (from 0 to 500 ms), delta ERS (0.5–3.5 Hz) in centroparietal regions is weaker in elderly subjects than in young, healthy controls (Xu et al., 2011). The latter researchers suggested that low delta ERS was related to greater involvement of cortical areas and thus greater recruitment of attentional resources during the cognitive task. In the present study, our observation of less delta/theta ERS in the donepezil condition means that more neurons were activated in parietal regions during the detection of the target stimulus (Steriade and Llinás, 1988; Pfurtscheller, 1992; Pfurtscheller et al., 1996). Acetylcholine is known to inhibit neural networks in the posterior parietal cortex; this inhibition may help to remove information from working memory and thus promote the more efficient processing of new

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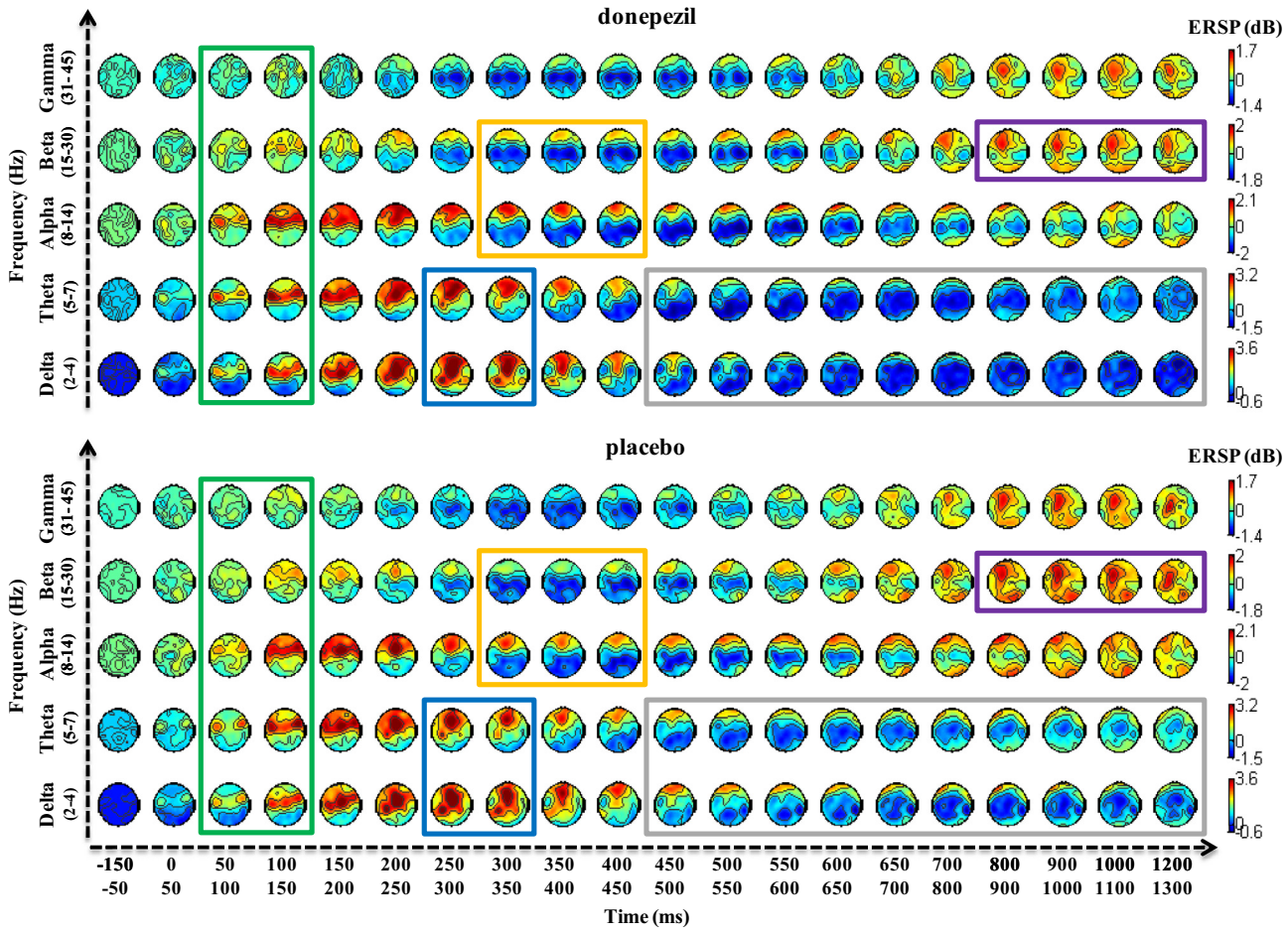

Fig. 4. Scalp ERSP topographies for the target stimulus. Each frequency band has its own color scale, which is the same in the donepezil and placebo conditions. Time windows (in milliseconds, ms) are shown on the *x*-axis and the frequency bands on the *y*-axis. Relative to the baseline activity, stronger power spectrum (in decibels, dB) (ERS) are shown in red and weaker power (ERD) are shown in blue.

information (Broussard, 2012). Moreover, it has been shown (in resting-state experiments) that donepezil can decrease delta activity in AD patients (Balkan et al., 2003). In the present study, the weaker delta/theta ERS in the donepezil condition for the target stimulus might have been linked to more efficient attentional processes following inhibition of the posterior parietal cortex. We also found that the parietal delta/theta ERS at about 300 ms was related to strong ITC (i.e. an evoked activity) for the target stimulus. In the auditory oddball paradigm, delta and theta ERSs are known to underlie the activity of the P300-ERP component (Basar-Eroglu and Demiralp, 2001; Mazaheri and Picton, 2005; Bernat et al., 2007). The frontal theta ERS arising at about 300 ms is known to be related to memory and attentional processes (Mazaheri and Picton, 2005; Missonnier et al., 2006). A magnetoencephalographic study of an auditory oddball paradigm has shown that the delta ERS in frontocentroparietal areas may be involved in auditory attention and memory updating processes (Ishii et al., 2009). In the present study, the classical analysis of P300 CSD-ERP (i.e. latency and amplitude) failed to highlight differences between donepezil and placebo conditions for the target stimulus. Time–frequency analyses based on CSD-ITC and -ERSP confirmed the literature results – namely that P300-ERP was present in the delta/theta frequency bands. Moreover, the inter-condition differences in delta/theta ERS at parietal electrodes suggested that attentional resources required for detection of the target stimulus also differed. The fact that a topography explaining at least 5% of variance did not emerge from the PCA analysis for the frequent stimulus (in the low frequency bands at about 300 ms; see the Supplementary Fig. S3B) confirms that the electrophysiological patterns observed for the target stimulus (i.e. delta/theta ERS at about 300 ms) might be linked to attentional processes in general and to P300 in particular. Furthermore, the differences observed for the target stimulus were located at left parietal electrodes (i.e. on the hemisphere contralateral to the response hand) at about the same time as the motor response (i.e. around 330 ms). An EEG study has already shown that theta ERS (4–7 Hz) over left frontal and parietal cortex (for right-handed subjects) has a role in movement-target detection (Rawle et al., 2012). In the present study, the left parietal delta/theta ERS for the target stimulus suggested a complex response combining motor component and cognitive processes, which differed when comparing the two conditions. This phase-locked delta/theta ERS may be associated with the resetting of prior auditory information processing (i.e. a rebound in initial cortical activation related to attention, and perception-motor processing); this would fit with P300's putative physiological role (i.e. cortical relaxation). Therefore, weaker delta/theta ERS in the

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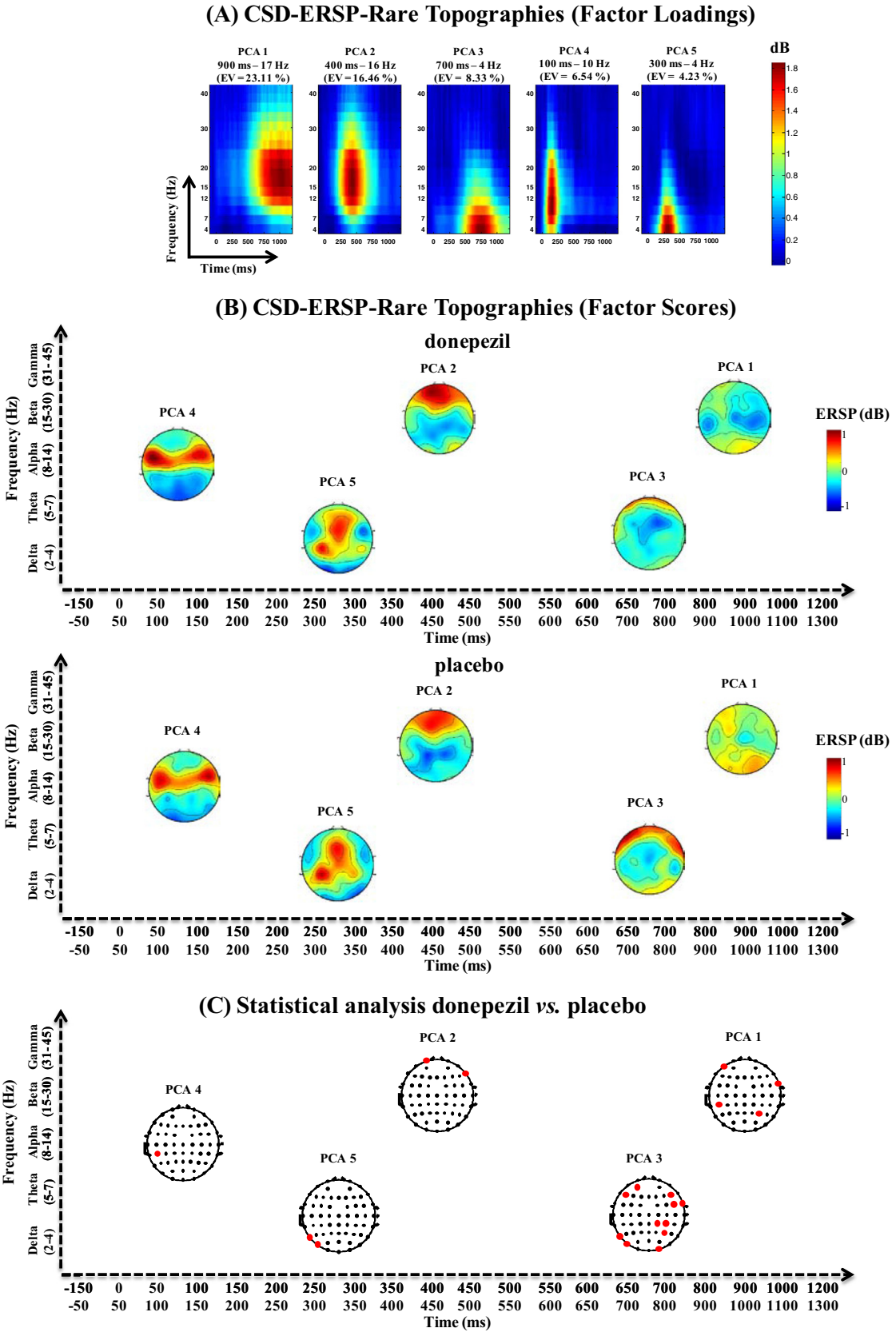

Fig. 5. CSD-ERSP results for the target stimulus in a PCA. (A) The five time–frequency maps correspond to the five eigenfunctions (factor loadings) that account for the most variance (i.e. the explained variance, EV: 58.67% of the total variance) in the ERSP data for the target stimulus. (B) For each eigenfunction, the CSD factor scores were pooled across all 24 participants for each condition and each electrode. Next, these values were used to display topographies in which ERS (in red) and ERD (in blue) are respectively indicated by a positive or negative sign for the factor score. (C) Topographies derived from the donepezil and placebo conditions were compared for each component in paired parametric Student's *t*-tests. Significant values ($p < 0.05$) were illustrated on a topographic map. Black dots show the electrode locations. A red dot indicates a significant difference between the two conditions for a given electrode.

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donepezil condition for the target stimulus is suggestive of prolonged, active auditory information processing (attention and perception) and sensorimotor transformation.

4.1.2. The alpha band

By performing a PCA on CSD-ERSP measurements during an auditory oddball task in young healthy subjects, Kayser et al. (2014) observed an ERS in the low-frequency band (delta/theta) at about 130 ms and at the centrotemporoparietal electrodes. This ERS was observed for both frequent and target stimuli and has been linked to N100-ERP. In our study, we found an alpha-band ERS at about 100 ms for target stimuli and a theta-/alpha-band ERS for frequent stimuli (see the Supplementary material, PCA 2 in Supplementary Fig. S3B). Our results are similar to those observed by Kayser et al. (2014) and suggest that these ERSs in theta/alpha bands (evoked activities) are associated with the N100 component. For the target stimulus, we also found a greater alpha ERS (at about 100 ms) at the left temporal electrode in the donepezil condition. For auditory stimuli, the N100-ERP component is generated by a neural network located in the primary and association temporal auditory cortices (Zouridakis et al., 1998; Godey et al., 2001). Alpha phase synchronicity is known to be involved in the generation of the N100 component (Haig and Gordon, 1998). Moreover, a study has already shown that alpha-phase locking values in left and right temporal regions were related to detection of the auditory stimulus in the human auditory cortex (Hsiao et al., 2009). In the present study, the observation of early differences in alpha ERS for the target stimulus suggested that donepezil improves the primary auditory processes (and particularly the detection of a target auditory stimulus) by increasing the acetylcholine concentration in frontotemporal regions (Bressler et al., 2008; Ko et al., 2012). This is corroborated by the results of animal studies. For example, in a study in the rat, increased acetylcholine release was observed in the frontal cortex and hippocampus during the detection of a new sensory stimulus (Acquas et al., 1996; Miranda et al., 2000). Furthermore, that donepezil improves auditory sensory gating mechanisms in the rat (Klinkenberg et al., 2013). Furthermore, a functional magnetic resonance imaging study of AD patients has shown that donepezil can improve perceptual responses and increase encoding abilities in working memory (Kircher et al., 2005). It has also been shown that the AChEI physostigmine can improve visual working memory performance in healthy volunteers by increasing the selectivity of neural responses in the visual cortex during the encoding phase (Furey et al., 2000). The cholinergic modulation that we observed in the alpha frequency band might be related to modifications of the selectivity of perceptual processing, which in turn would result in changes in working memory performance (Furey et al., 2000). Thus, donepezil might increase selectivity and efficiency of neural information processing, with greater inhibition of irrelevant information processing and cortical activity. This might result in less cortical activation and equivalent behavioral performance (when comparing the donepezil and placebo conditions).

4.2. Induced activities

4.2.1. The delta and theta bands

At about 700 ms (for the target stimulus), a delta/theta ERS related to an induced activity was weaker in the donepezil condition – especially at the frontal electrodes. An increase in acetylcholinesterase inhibition in frontal regions (the anterior cingulate cortex, and dorsolateral prefrontal and posterior cingulate cortices) has been observed by positron emission tomography in donepezil-treated AD patients (Bohnen et al., 2005). Otherwise, assessment of an auditory oddball paradigm in the rat showed that lesions of the nucleus basalis of Meynert were associated with greater delta/theta ERS in the frontal cortex (from 100 ms to 700 ms after the onset of the target stimulus) (Sanchez-Alavez et al., 2014). In the present study, the weaker frontal delta/theta ERS in the donepezil condition suggests the blockage of rhythmic EEG components in the low-frequency bands – reflecting a cortical activation state (Steriade et Llinás, 1988). These effects might be related to the drug's inhibition of acetylcholinesterase. However, this delta/theta ERS appeared in regions near to eyes and had a long latency relative to the onset of the target stimulus. It is possible that these activities correspond to low-amplitude eye movements (Cordones et al., 2013). If so, this would explain why these electrophysiological patterns are also observed for the frequent stimulus in the donepezil and placebo conditions (see the Supplementary material, PCA 4 in Supplementary Fig. S3B). Alternatively, these factor score topographies (for target and frequent stimuli) may reflect ERD that is widely distributed over the scalp after a decrease in alertness has reactivated slow rhythms.

4.2.2. The alpha band

For the target stimulus, an alpha/beta ERD was observed at the centroparietal electrode – especially on the left side and at about 400 ms. Alpha ERD is related to the P300 wave (Peng et al., 2012) and is known to reflect attentional and memory processes during an oddball paradigm (Klimesch, 1999). Another recently study using an auditory oddball task in healthy young adults has shown that CSD-ERSP measures are suitable for highlighting alpha ERD (350–600 ms) for the target stimulus. This ERD has been linked to a decrease with respect to the prestimulus baseline (Tenke et al., 2015). Moreover, by performing a PCA on CSD-ERSP data from healthy young adults, Kayser et al. (2014) found an alpha ERD (9 Hz) after about 600 ms at the centroparietal electrodes. This ERD was observed for target stimulus but not for the frequent stimulus (Kayser et al., 2014). The present study used the same methodology (i.e. a PCA of CSD-ERSP data) and found similar electrophysiological patterns in the donepezil and placebo conditions for both the frequent stimulus (see the Supplementary material, PCA 3 in Supplementary Fig. S3B) and the target stimulus. Furthermore, we did not find a statistically significance inter-condition difference for the ERD after the target stimulus. Event-related oscillations in the alpha band are thought to reflect inhibitory cortical control processing (Uhlhaas and Singer, 2010). More particularly, alpha ERD corresponds to the release of cortical inhibition and the synchronized activation of cortical networks that are dependent on the cognitive task (Klimesch et al., 2007). In the present study, the similarity of the alpha ERD in both conditions for the target stimulus suggests that donepezil does not recruit more neurons for promoting more efficient attentional mechanisms. In other words, donepezil does not seem to modulate top-down processing (Koh et al., 2011).

Furthermore, alpha ERD may be also associated with activation of the cortical areas involved in sensory processing and the production of motor behavior (Pfurtscheller, 1992). The presence of left-lateralized alpha ERD for the right-handed responses just after the motor response (for the target stimulus) corroborates this hypothesis. It has been established that acetylcholine blocks slow oscillations and contributes to the desynchronization of EEG rhythms (Steriade, 1993). Moreover acetylcholine axons project from the basal forebrain to the thalamus and neocortex (Mesulam, 2004). Given that induced activities are reflected by thalamocortical interactions, it is therefore surprising that the donepezil condition was not associated with greater alpha ERD for the target stimulus.

4.2.3. The beta band

For the target stimulus, induced activity (reflected by a beta ERS at frontal electrodes at about 400 ms) was stronger in the

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donepezil condition. This activity was observed just after the motor response (i.e. at about 330 ms). It is well accepted that beta activities are related to the motor response (Pfurtscheller et al., 1998; Houdayer et al., 2006; Jurkiewicz et al., 2006). Moreover, acetylcholine may have a role in movement because many cholinergic afferents from the nucleus basalis of Meynert project to the entire cortex (including the motor cortex) (Conner et al., 2003; Berg et al., 2005). Alternatively, an increase in beta ERS for a target stimulus might be related to more efficient high-level cognitive functions (such as attention) (Güntekin et al., 2013). However, conflicting results have been reported. Some EEG studies of attentional paradigms have observed beta ERS (Onton et al., 2005; Kukleta et al., 2009), whereas others have observed beta ERD (Cacace and McFarland, 2003; Mazaheri and Picton, 2005).

Although the factor score topography for the donepezil condition was not easy to interpret, our ERSP analyses appear to evidence several induced activities in the beta band at about 900 ms for the target stimulus. Beta ERS at the frontal and parieto-occipital electrodes was weaker in the donepezil condition. Conversely, beta ERD at the temporoparietal electrodes was greater in the donepezil condition (at about 900 ms). A study of an auditory oddball task showed that late beta ERS in frontotemporal regions (from 750 to 1000 ms) and occipital regions (from 500 to 750 ms) was significantly weaker in patients with schizophrenia than in young, healthy subjects (Fujimoto et al., 2012). The latter researchers also observed stronger beta ERD (from 750 to 1000 ms) in the posterior frontal, temporal and parietal regions in patients with schizophrenia. However, in this previous study, all the patients were being treated with antipsychotic drugs, the effects of which might also have contributed to changes in neural oscillations (Fehr et al., 2001). Alterations of functional brain connectivity in large-scale networks have been observed in patients with schizophrenia (Uhlhaas, 2013). Furthermore, reductions in the amplitude of neural oscillations and lower phase synchronization in induced oscillatory activity have been observed in patients with schizophrenia during cognitive tasks (Uhlhaas and Singer, 2010). Other studies of patients with schizophrenia have shown a reduction in phase synchronization in the beta band during a visuoperceptive task (Uhlhaas et al., 2006). Although it is not easy to compare the electrophysiological patterns of healthy subjects with those of patients, our present results suggest that ERS and ERD patterns in donepezil-treated healthy subjects for the target stimulus are associated with modulation of neuronal synchronization in various cortical networks. However, these late beta activities occurred almost 1000 ms after the onset of the target stimulus, and so this hypothesis remains to be confirmed. In contrast to the present study, the auditory oddball paradigm studied by Fujimoto et al. (2012) did not require a motor response. The beta ERS/ERD patterns observed in the present study might also be related to the so-called post-movement beta rebound, which is related to the resetting of cortical information processing of movement-related somatosensory afferents (Pfurtscheller and Lopes da Silva, 1999; Houdayer et al., 2006; Jurkiewicz et al., 2006). Given that donepezil reduced the induced frontal-parieto-occipital beta ERS at about 900 ms for the target stimulus, we assume that it prolonged active information processing of somatomotor afferents. The increase in the induced temporoparietal beta ERD in the donepezil condition for the target stimulus also suggests that the drug prolonged active information processing of somatosensory afferents.

4.2.4. The gamma band

Our PCA did not identify relevant CSD-ERSPs in the gamma band for either the target stimulus or the frequent stimulus (see the Supplementary material, Supplementary Fig. S3A). We chose to analyze this frequency band because an EEG study of an auditory oddball task had shown that gamma band oscillatory responses were strongly correlated with the P300 wave (Gurtubay et al., 2001). Moreover, cholinergic modulation has an important role in gamma oscillations and response synchronization (Rodriguez et al., 2004).

4.3. Study limitations

In the present study, we sought to identify dynamic EEG markers in young healthy adults. This population was chosen in order to reduce the age-related inter-individual variability in cognitive functioning. However, AD is a disease that occurs mainly in older adults, and so our study should be replicated in healthy elderly adults. Otherwise, some of the results described above were based on data from border electrodes (i.e. frontal, temporal and occipital electrodes). It is known that spline Laplacian estimations may be erroneous for border electrodes (Babiloni et al., 1995). Therefore, one must interpret these results with caution. Use of a larger number of scalp electrodes (up to 256) could significantly reduce these estimation errors (Babiloni et al., 1995). Furthermore, the present study used a spherical spline Laplacian computed with a moderately intermediate flexible spline ($m = 4$). It was recently shown that a spherical spline Laplacian computed with rigid splines ($m \geqslant 5$) can maintain activity at border electrodes (Tenke and Kayser, 2015). Another limitation concerned our choice of methodology (an oddball paradigm that required a motor response). The interpretation of some of our results was complicated by the fact that it is difficult to distinguish between EEG activities related to cognitive processes and those associated with the motor finger response. It would be interesting to study a paradigm in which participants are asked to mentally count the target stimuli (Fujimoto et al., 2012).

4.4. Conclusion

CSD transformations of the ERSP and ITC data revealed significant differences between the donepezil and placebo conditions in young, healthy, adult participants. Whereas the CSD-P300 ERP and behavioral analyzes did not reveal any inter-condition differences, the evoked activities observed in the time–frequency domain (i.e. ERSP and ITC) for the target stimulus showed that donepezil modulates (i) delta/theta ERS underlying the P300 waveform and (ii) alpha ERS involved in generation of the N100 waveform. Our ERSP data also revealed that donepezil was associated with some late changes (with respect to the onset of the target stimulus) in induced activities in the delta/theta (ERS) and beta (ERS/ERD) frequency bands for the target stimulus.

Lastly, the present study identified a number of group-level EEG biomarkers of donepezil's effect on young, healthy, adult volunteers. There is a need to develop AD drugs that are more effective than donepezil. EEG markers generated by CSD-ERSP and CSD-ITC analyses show that electroencephalography might be a useful additional tool for assessing the therapeutic potential of drug candidates in AD prior to Phase II/III clinical trials. However, the present study needs to be replicated so that the EEG methods described here can be validated for use in drug trials.

Acknowledgements

The present study was funded by the European Union's Seventh Framework Programme (FP7: 2007–2013) as part of the Innovative Medicine Initiative (grant agreement 115009: prediction of cognitive properties of new drug candidates for neurodegenerative diseases in early clinical development, PharmaCog). For more information about the PharmaCog project, please refer to http://www.pharmacog.org.

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Conflict of interest: The authors have no conflict of interests to declare.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinph.2015.11.018.

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