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Donepezil effects on sources of cortical rhythms in mild Alzheimer's disease: Responders vs. Non-Responders

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Acetylcholinesterase inhibitors (AChEI) such as donepezil act in mild Alzheimer's disease (AD) by increasing cholinergic tone. Differences in the clinical response in patients who do or do not benefit from therapy may be due to different functional features of the central neural systems. We tested this hypothesis using cortical electroencephalographic (EEG) rhythmicity. Resting eyes-closed EEG data were recorded in 58 mild AD patients (Mini Mental State Examination [MMSE] range 17–24) before and approximately 1 year after standard donepezil treatment. Based on changes of MMSE scores between baseline and follow-up, 28 patients were classified as "Responders" (MMSEvar $\geq$0) and 30 patients as "Non-Responders" (MMSEvar <0). EEG rhythms of interest were delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10.5 Hz), alpha 2 (10.5–13 Hz), beta 1 (13–20 Hz), and beta 2 (20–30 Hz). Cortical EEG sources were studied with low-resolution brain electromagnetic tomography (LORETA). Before treatment, posterior sources of delta, alpha 1 and alpha 2 frequencies were greater in amplitude in Non-Responders. After treatment, a lesser magnitude reduction of occipital and temporal alpha 1 sources characterized Responders. These results suggest that Responders and Non-Responders had different EEG cortical rhythms. Donepezil could act by reactivating existing yet functionally silent cortical synapses in Responders, restoring temporal and occipital alpha rhythms.

Keywords: Mild Alzheimer's disease (mild AD); Donepezil; Electroencephalography (EEG); Alpha rhythm; Low resolution brain electromagnetic tomography (LORETA)

Introduction

Acetylcholinesterase inhibitors (AChEI) act by opposing the cholinergic transmission deficit in Alzheimer's disease (AD; Geula and Mesulam, 1999; Mesulam, 2004). First-generation AChEI such as tetrahydroaminoacridine (THA) and physostigmine, because of poor central nervous system selectivity, had major side effects (Kogan et al., 2001; Summers et al., 1986; Stern et al., 1988; Farlow et al., 1992) and THA also caused hepatotoxicity in many patients (Farlow et al., 1992). Second-generation AChEI such as donepezil, rivastigmine, and galantamine, instead, have been effective and well tolerated (Burns et al., 1999; Kogan et al., 2001; Farlow, 2002; Birks and Harvey, 2003; Brassen and Adler, 2003; De La Garza, 2003; Geldmacher et al., 2003; Lanctot et al., 2003; Relkin et al., 2003; Rodriguez et al., 2002, 2004). A positive response occurs on average in 50% of treated AD patients, considered "Responders" (Tanaka et al., 2003; Hanyu et al., 2003a; Bianchetti et al., 2003; Onofrj et al.,

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2003, Jones, 2003), even though some authors estimate true Responders to be 10% only once placebo effects are removed (Lanctot et al., 2003).

The cause of this limited response is unclear. To date, Responders and Non-Responders have been found to differ in anatomical features (Tanaka et al., 2003) and in the patterns of regional cerebral blood flow (rCBF, Hanyu et al., 2003a). An important issue is the determination of the specific relationships between treatment and cholinergic systems in AD Responders.

Converging evidence supports the hypothesis of a relationship between brain cholinergic transmission and spontaneous electroencephalographic (EEG) rhythms in resting and awake conditions. These rhythms would reflect bi-directional connections among several cortical and sub-cortical (i.e. brainstem, thalamus) structures and their post-synaptic targets in the cortical pyramidal layers (Pfurtscheller and Lopez da Silva, 1999).

Animal studies have shown that EEG rhythmicity changes with the manipulation of cholinergic tone (Holschneider et al., 1998; Mesulam, 2004). In humans, EEG rhythms differ in AD patients compared to normal controls and/or vascular dementia subjects. AD patients were characterized by higher delta (0–3 Hz), higher theta (4–7 Hz), lower posterior alpha (8–12 Hz), slowing in alpha peak frequency, and lower beta (14–30 Hz) and gamma (around 40 Hz) (Dierks et al., 1993, 2000; Huang et al., 2000; Wolf et al., 2003; Babiloni et al., 2004; Moretti et al., 2004; Adeli and Ghosh-Dastidar, 2005). The abnormality of EEG rhythms in dementia has been associated with altered rCBF/metabolism and cognitive function (Szelies et al., 1992; Julin et al., 1995; Passero et al., 1995; Sloan et al., 1995; Rodriguez et al., 1999a, 1999b). Furthermore, parieto-temporal EEG rhythms and rCBF have been correlated with severity of AD as measured by Mini Mental State Evaluation scores (MMSE).

Second-generation AChEI have been found to restore near normal EEG rhythmicity in mild AD patients. In particular, short-term treatment induced theta decrement (4–7 Hz; Brassen and Adler, 2003), alpha increment (8–12 Hz; Onofrj et al., 2003), as well as alpha and delta (0–3 Hz) decrement (Reeves et al., 2002). Long-term treatment, instead, induced decrement of theta (Kogan et al., 2001) and increment of alpha/theta ratio especially in the frontal regions (Rodriguez et al., 2002). Finally, the mean power across several frontal and posterior rhythms has been proven to be not sensitive to the long-term effects of treatment in mild to moderate AD patients (Rodriguez et al., 2004), possibly due to limitations of the standard EEG approach used.

In the present study, resting EEGs were recorded in mild AD patients before and approximately 1 year after standard donepezil treatment (5–10 mg/day). Sources of EEG rhythmicity were studied with the technique called low-resolution brain electromagnetic tomography (LORETA) (Pascual-Marqui and Michel, 1994), successfully used in recent EEG studies on aging (Dierks et al., 2000; Babiloni et al., 2004, 2005a,b,c,d). Principal aims of the present study were: i) mapping of EEG source differences between Responders and Non-Responders before treatment, as a baseline for future studies on predictors of therapy efficacy, and ii) evaluation of the treatment effects on EEG sources in Responders, to understand the contribution of augmented cholinergic tone on cortical rhythmicity. A comparison between AD patients taking donepezil or a placebo, though ideal, was not attempted mostly because of ethical constraints.

Methods

We have extensively described in recent papers on EEG and aging most of the procedures (subjects recruitment procedures, EEG recordings, LORETA analysis, statistics) pertinent to the current study (Babiloni et al., 2004, 2005a,b,c,d). They described the results of an international multi-centric EEG study on aging involving several neurological research units. It should be remarked that none of those papers addressed the specific aim of the present study and focused on the long-term effects of donepezil on cortical EEG rhythms in AD subjects who responded vs. not responded to the therapy. Therefore, the results of the present study are totally novel and unedited. For the convenience of readers, here, we described the EEG methodology even if it was reported in the mentioned previous papers.

Subjects

For the present multi-centric study, 58 mild AD patients were enrolled. As controls, 65 cognitively normal elderly subjects (Nold) were also recruited. The study was approved by the local institutional ethics committees. All experiments were undertaken with the understanding and written informed consent of each participant or caregiver, according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the Author's Institutional Review Board.

Diagnostic criteria

Probable AD was diagnosed according to NINCDS-ADRDA (McKhann et al., 1984) and DSM IV criteria. AD patients underwent general medical, neurological and psychiatric assessments. They were also rated with a number of standardized diagnostic and severity instruments that included the MMSE (Folstein et al., 1975), Clinical Dementia Rating Scale (CDR; Hughes et al., 1982), Geriatric Depression Scale (GDS, Yesavage et al., 1982–1983), Hachinski Ischemic Scale (Rosen et al., 1980), and Instrumental Activities of Daily Living (IADL, Lawton and Brodie, 1969). In addition, they underwent neuroimaging diagnostic procedures (CT or MRI) and complete laboratory analysis. The main objective of the diagnostic phase was the exclusion of other types of progressive or reversible dementias in order to have a homogenous AD patient sample that could be divided between "Non-Responders" and "Responders" to the donepezil therapy. In particular, exclusion criteria were evidence of concomitant (i) extra-pyramidal symptoms, (ii) reversible dementias (including pseudo-depressive dementia), (iii) fluctuations in cognitive performance (suggesting a possible Lewy body dementia), and/or features of mixed dementias (including AD plus vascular dementia).

Diagnostic criteria

In the AD patients, MMSE scores were obtained before (baseline I MMSE) and approximately after 1 year (follow-up II MMSE; 11.3 months ± 2.6 standard deviation, SD) of the donepezil treatment (5–10 mg/day). Before treatment, the AD patients had MMSE scores ranging from 17 to 24. Based on the difference of MMSE scores between baseline and follow-up (MMSEvar), 28 patients were classified as Responders (MMSEvar $\geq$0) and 30 patients as Non-Responders (MMSEvar <0). This

criterion focused on a "global" rather than a "cognitive" response since clinically relevant changes reflecting a positive therapeutic outcome are not always captured on strictly cognitive scales (Mayeux and Sano, 1999; Pryse-Phillips, 1999). The Responders included patients showing an increase of MMSE score during therapy (MMSEvar ≥ 0), based on the common definition of AD as an irreversible, progressive disorder characterized by neuronal deterioration that results in loss of cognitive functions (Lanctot et al., 2003). This definition implies a certain loss of global cognitive functions after 1 year of the disease. The criterion used was quite useful in our study since it divided the AD patients in two nearly equal halves with similar features. There was indeed no statistical ANOVA difference ($P > 0.05$) in baseline MMSE scores (I MMSE) between Responders (21.4 ± 3.9 SD) and Non-Responders (21.3 ± 2.8 SD). Similarly, no statistical ANOVA difference ($P > 0.05$) was present for the mean donepezil treatment duration in Responders (10.8 months ± 1.9 SD) and Non-Responders (11.9 months ± 3.2 SD).

A control group of healthy elderly subjects (Nold) was recruited mainly among patients' partners. All Nold subjects underwent physical and neurological examinations as well as cognitive screening (including MMSE and GDS). Subjects affected by chronic systemic illnesses (e.g. diabetes mellitus) were excluded, as were subjects receiving psychoactive drugs. Subjects with a history of present or previous neurological or psychiatric disease were also excluded.

Details on the features of recruited subjects are provided in Tables 1 and 2. Table 1 reports the mean values of relevant personal and clinical characteristics of participating mild AD and Nold. Table 2 reports the means of the personal and clinical characteristics of participating mild AD patients, subdivided in Responders and Non-Responders. Of note, the difference in age and education between the two AD sub-groups was negligible. These variables were nonetheless used as covariates in the statistical evaluation of cortical sources of EEG rhythms.

EEG recordings

EEG was recorded (Neuroscan, EB-Neuro, or Micromed EEG machines) in resting subjects (eyes closed) by specialized personnel of the clinical units (Brescia, A.Fa.R.-Rome, Campus Biomedico Rome, Troina, Genova). EEG data were acquired (0.3–70 Hz bandpass) from 19 electrodes positioned according to the international 10–20 system (i.e. Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2). A specific kind of reference electrode was not used in all recording units, given that the present preliminary data analysis and LORETA source analysis were based on common average reference. To monitor eye movements, the electrooculogram (0.3–70 Hz bandpass) was also recorded. All data were digitized in continuous recording mode (5 min of EEG; 128–256 Hz sampling rate, the sampling rate being fixed in each recording research unit of this multi-centric study; data analysis was performed at 128 Hz). It is noteworthy that the EEG data were recorded before (recording I) and about after 1 year (recording II) of donepezil treatment in the AD patients. In all subjects, EEG recordings were performed in the late morning. In order to maintain vigilance, an operator monitored each subject clinically and by inspecting the EEG traces on-line, verbally alerting the subject any time behavioral and/or EEG signs of drowsiness appeared.

Table 1
Personal and neuropsychological data of interest of normal elderly controls (Nold) and mild Alzheimer's disease subjects (AD)

	Nold	Mild AD
N	65	58
Age (years)	72.1 (±7.1 SD)	77.1 (±6.7 SD)
Education (years)	9.5 (±4.3 SD)	5.7 (±3.1 SD)
MMSE	28.2 (±1.2 SD)	21.4 (±3.4 SD)
Gender (M/F)	29/36	14/44

Table 2
Personal and neuropsychological data of interest of the Alzheimer's disease (AD) subjects, subdivided in Responders (II MMSE–I MMSE > 0) and Non-Responders (II MMSE–I MMSE < 0)

	Non-Responder	Responder
N	28	30
Age (years)	77.7 (±6.4 SD)	76.6 (±7.1 SD)
Education (years)	4.9 (±2.6 SD)	6.6 (±3.3 SD)
I MMSE	21.4 (±3.9 SD)	21.3 (±2.8 SD)
II MMSE	18.6 (±4.5 SD)	23.2 (±2.8 SD)
II MMSE–I MMSE	−2.8 (±2.6 SD)	+1.8 (±1.4 SD)
Gender (M/F)	8/20	6/24

Legend: I MMSE = MMSE before donepezil treatment, II MMSE = MMSE after 1 year of donepezil treatment.

Of note, EEG recordings lasting 5 min allow the comparison of the current results with several previous AD studies also using recording EEG periods not longer than 5 min (Buchan et al., 1997; Pucci et al., 1999) or even shorter than 1 min (Dierks et al., 1993, 2000). In particular, this protocol has been successful in a previous reference study of long-term donepezil effects on scalp EEG in AD patients (Rodriguez et al., 2002). Longer resting EEG recordings in AD patients could have reduced the data variability but would have increased the possibility of EEG slowing and oscillations due to reduced vigilance and arousal.

The EEG data were analyzed and fragmented off-line in consecutive epochs of 2 s. On average, 116 epochs for each subject were examined. For standardization purposes, the analysis of all data was performed at the EEG laboratories of the Dept. of Human Physiology and Pharmacology in Rome. The EEG epochs with ocular, muscular, and other types of artifact were preliminarily identified by a computerized automatic procedure. The EEG epochs including ocular artifacts (less than 15% of the total ones) were then corrected by an autoregressive method (Moretti et al., 2003). Finally, two independent experimenters manually confirmed the EEG segments accepted for further analysis. Of note, at the time of the EEG analysis, the experimenters were blind to the classification of patients in the groups of Responders and Non-Responders (the classification was based on clinical information gathered after 1 year of treatment).

Spectral analysis of the EEG data

A digital FFT-based power spectrum analysis (Welch technique, Hanning windowing function, no phase shift) computed the power density of EEG rhythms with 0.5 Hz frequency resolution. The following standard band frequencies were studied: delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10.5 Hz), alpha 2 (10.5–13 Hz), beta 1 (13–20 Hz), and beta 2 (20–30 Hz). These band frequencies were chosen averaging those used in previous relevant EEG studies on dementia in order to render comparable present results with them (Leuchter et al., 1993; Jelic et al., 1996; Besthorn et al., 1997;

Chiaramonti et al., 1997; Rodriguez et al., 1999a, 1999b) and with previous findings of our Consortium (Babiloni et al., 2004, 2005b,c,d,e). This advantage was supposed to compensate the use of odd 10.5 Hz as half-limit between alpha 1 and alpha 2 bands.

Sharing of a frequency bin by two contiguous bands has been shown to be adequate by much EEG literature (Cook and Leuchter, 1996; Jelic et al., 1996; Besthorn et al., 1997; Holschneider et al., 1999). Furthermore, it follows the theoretical consideration of Klimesch and others stating that near EEG rhythms may overlap at their frequency borders (see Klimesch, 1999 for a review). This choice made more significant the between-groups source differences at a given band but not at the contiguous ones. However, it should be noted that the choice of fixed bands did not account for individual EEG markers such as individual alpha and transition frequencies (Klimesch, 1999). Furthermore, we could not use narrow frequency bands for beta 1 (13–20 Hz) and beta 2 (20–30 Hz) because of the variability of the beta peaks in the power spectra. Therefore, the LORETA results for the beta bands could suffer from the sensitivity limitations of EEG spectral analyses for large bands (Szava et al., 1994).

Choice of fixed EEG bands did not account for individual alpha frequency (IAF) peak, defined as the frequency associated with the strongest EEG power at the extended alpha range (Klimesch, 1999). To evaluate the possible effects of this factor, we computed the IAF for our subjects. In the two AD sub-groups, the mean IAF peaks were 8.7 (±0.2 SE) for Responders and 8.6 (±0.3 SE) for Non-Responders. Therefore, the IAF factor clearly could not account for possible differences between EEG sources in Responders vs. Non-Responders, namely the scientific issue of the present study.

Cortical source analysis of the EEG rhythms by LORETA

As previously mentioned, the LORETA technique was used for the EEG source analysis provided at http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm (Pascual-Marqui and Michel, 1994). LORETA computes 3-D linear solutions (LORETA solutions) for the EEG inverse problem within a three-shell spherical head model including scalp, skull, and brain compartments. The brain compartment is restricted to the cortical gray matter/hippocampus of a head model co-registered to the Talairach probability brain atlas and digitized at the Brain Imaging Center of the Montreal Neurological Institute (Talairach and Tournoux, 1988). This compartment includes 2394 voxels (7 mm resolution), each voxel containing an equivalent current dipole.

LORETA solutions consisted of voxel current density values able to predict EEG spectral power density at scalp electrodes. The idea that LORETA is a reference-free method of EEG analysis is widely accepted in that one obtains the same LORETA source distribution for EEG data referenced to any reference electrode including a common average. Normalization was obtained by normalizing the LORETA current density at each voxel for the LORETA power density averaged across all frequencies (0.5–45 Hz) and voxels of the brain volume. The general procedure fitted the LORETA solutions in a Gaussian distribution and reduced inter-subject variability (Nuwer, 1988; Leuchter et al., 1993). Of note, other methods of normalization using the principal component analysis are effective for estimating the subjective global factor scale of the EEG data (Hernández et al., 1994). These methods are not available in the LORETA package, so they were not used in this study.

Solutions of the EEG inverse problem are underdetermined and ill-conditioned when the number of spatial samples (electrodes) is lower than that of the unknowns (current density at each voxel), as is the case of the present study (i.e. 19 EEG electrodes and 2394 voxels). To account for that, cortical LORETA solutions predicting scalp EEG spectral power density were regularized to estimate the distributed rather than the punctual EEG sources (Pascual-Marqui and Michel, 1994). This provides the maximally smoothed cortical source patterns explaining scalp EEG that characterize the LORETA technique and its name (i.e. low-resolution brain electromagnetic tomography). To further reduce the spatial resolution in line with the low number of electrodes used, we collapsed the LORETA solutions at frontal, central, temporal, parietal, and occipital regions of the brain model coded into Talairach space. Brodmann areas listed in Table 3 formed each one of these ROIs. When compared to other approaches to punctual EEG source analysis (i.e. dipole localization, L1-norm linear inverse estimation), such spatial smoothing of the LORETA solutions (resolution in centimeters) could reliably take into account for the slight change in the cortical volume (resolution in millimeters) present in the mild stages of AD (note that both Responders and Non-Responders were recruited at an early disease stage and they had similar age, disease duration, and MMSE scores at baseline recordings). Keeping these considerations in mind, it is unlikely that the differences in LORETA solutions between the groups (Responder, Non-Responder) depended on differences in brain atrophy, although such changes can be crucial from a functional point of view as demonstrated in subjects with mild cognitive impairment (Visser et al., 1999). Indeed, it is a widely accepted procedure – within the LORETA frame – to use a template head model in the investigation of EEG rhythms in physiological and pathological aging (Anderer et al., 1998a,b, 2003; Dierks et al., 2000; Huang et al., 2002; Saletu et al., 2002; Goforth et al., 2004; Babiloni et al., 2004; Cincotti et al., 2004).

With respect to the spectral analysis of scalp EEG data, the present LORETA analysis may better disentangle EEG rhythms of contiguous cortical areas by explicit lobar source modeling, e.g. parietal vs. occipital EEG sources. Furthermore, the use of explicit lobar EEG sources into a Talairach space may facilitate multimodal comparisons with structural and functional neuroimaging on aging (SPECT, PET, SPECT).

Statistical analysis of the LORETA solutions

To evaluate the Gaussianity of the regional normalized LORETA solutions, a preliminary Kolmogorov–Smirnoff analysis

Table 3
Brodmann areas included in the cortical regions of interest (ROIs) of this study

LORETA	Brodmann areas into the regions of interest (ROIs)
Frontal	8, 9, 10, 11, 44, 45, 46, 47
Central	1, 2, 3, 4, 6
Parietal	5, 7, 30, 39, 40, 43
Temporal	20, 21, 22, 37, 38, 41, 42
Occipital	17, 18, 19

LORETA solutions were collapsed in frontal, central, parietal, temporal, and occipital ROIs.

was performed on the data of mild AD subjects ($P < 0.05$). The results showed that most of the mentioned LORETA solutions were normally distributed ($P < 0.05$). The unique exceptions were temporal alpha 2 and occipital beta 1 LORETA solutions in mild AD patients. These LORETA solutions were not further considered in the following parametric analyses.

Regional normalized LORETA solutions from mild AD subjects were used by ANOVA. Subjects' age and education served as covariates. Mauchly's test evaluated the sphericity assumption. Correction of the degrees of freedom was made with the Greenhouse–Geisser procedure. Duncan test was used for post hoc comparisons ($P < 0.05$). In particular, two ANOVA designs were used to address the main scientific issues of the study.

The statistical design of the first ANOVA was focused on the hypothesis of possible EEG differences of Responders and Non-Responders at the recording I and recording II. This hypothesis implied the use of EEG data of the recording I and recording II as a dependent variable. The ANOVA factors (levels) were Group (Responders, Non-Responders; independent variable), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). To stress the possible predictive value of the EEG source analysis, EEG sources characterizing Responders with respect to Non-Responders before treatment were correlated with the MMSE score variation during therapy. In particular, the correlation between the first EEG LORETA relative current density values and the difference in MMSE scores (II MMSE–I MMSE) in the AD groups was computed with the Pearson test ($P < 0.05$).

The statistical design of the second ANOVA was focused on the hypothesis of possible EEG differences across 1 year between Responders and Non-Responders. This implied the use of EEG differences between recordings I and II (recording II minus recording I) as a dependent variable. The ANOVA factors (levels) were Group (Responder, Non-Responder; independent variable), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). As a note, the two mentioned ANOVAs could not be fused into a unique ANOVA since they had different dependent variables.

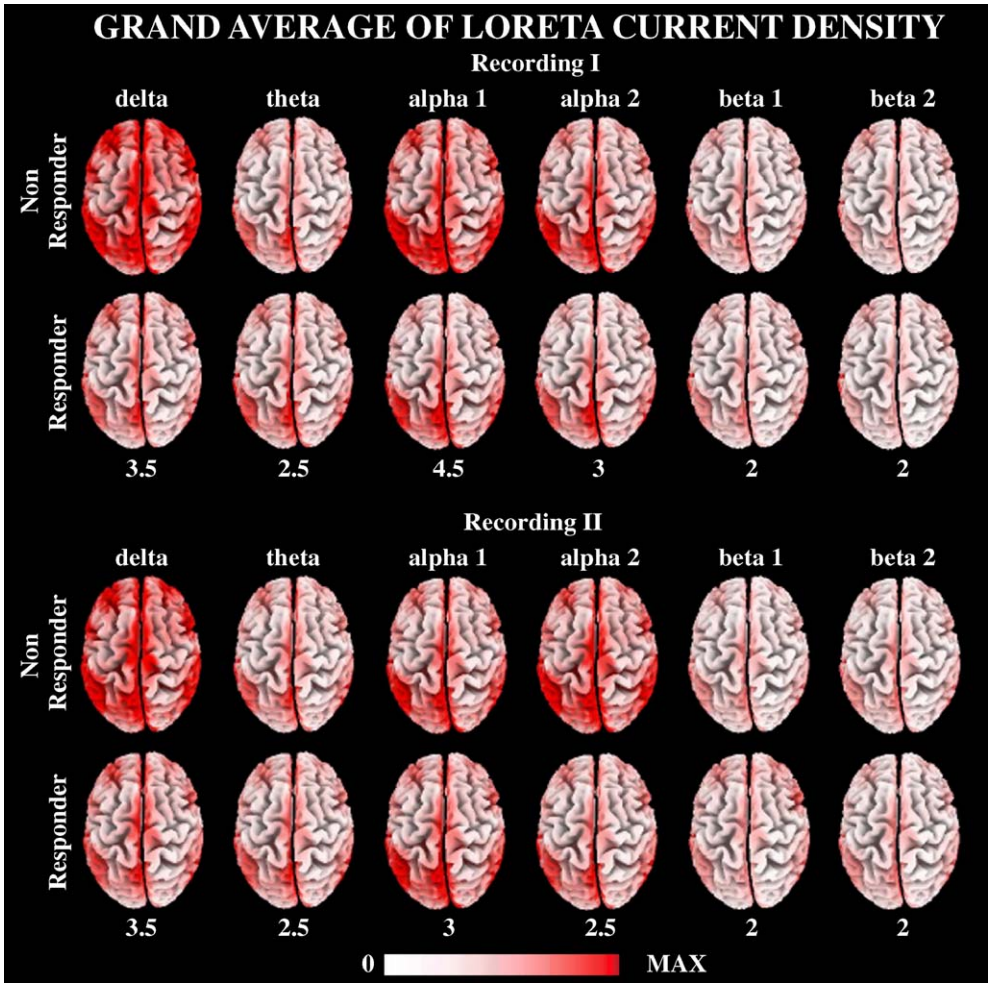

Fig. 1. Grand average of LORETA solutions (i.e. relative current density at cortical voxels) modeling distributed EEG sources for delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10.5 Hz), alpha 2 (10.5–13 Hz), beta 1 (13–20 Hz), and beta 2 (20–30 Hz) bands in mild AD Responders (II MMSE–I MMSE > 0) and Non-Responders (II MMSE–I MMSE < 0) during recording I and II. The left side of the maps (top view) corresponds to the left hemisphere. Color scale: all power estimates were scaled based on the averaged maximum value (i.e. alpha 1 power value of occipital region in Non-Responder). The maximal value of power is reported.

Results

Topography of the EEG cortical sources estimated by LORETA

For illustrative purpose, Fig. 1 maps the grand average of LORETA solutions, modeling the distributed EEG sources for delta, theta, alpha 1, alpha 2, beta 1, and beta 2 bands in Responders and Non-Responders at recordings I and II. Compared to Non-Responders, the Responder group was characterized by delta, alpha 1, and alpha 2 sources that were lower in magnitude before the donepezil treatment (recording I), and by delta and alpha 2 sources lower in magnitude after treatment (recording II). Of note, there were differences in the sources of delta and alpha rhythms in the two AD groups but not in those of theta rhythms

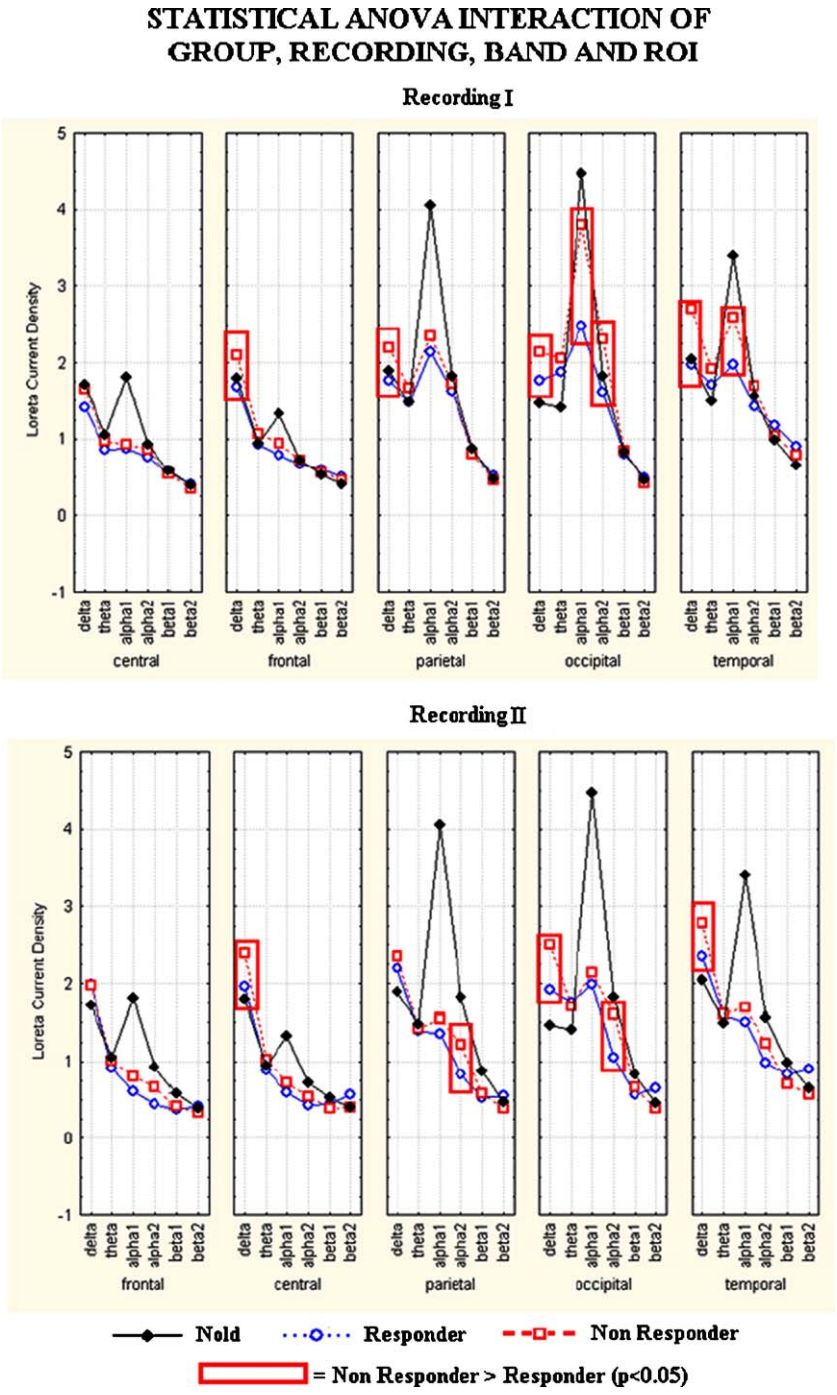

Fig. 2. Regional LORETA solutions (distributed EEG sources) relative to a statistical ANOVA interaction among factors Group (Non-Responder, Responder), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). This ANOVA focused on the features of the EEG sources characterizing Responders with respect to Non-Responders before (top) and after (bottom) 1 year of donepezil treatment. Legend: rectangles indicate the cortical regions and frequency bands in which LORETA solutions presented statistically different values ($P < 0.05$, Duncan post hoc test) in Non-Responder with respect to Responder subjects.

(note that theta rhythms increase in magnitude during very early sleep onset). These findings indicate comparable levels of vigilance for Responder and Non-Responder groups during EEG recordings.

Regional LORETA solutions characterizing Responders compared to Non-Responders

Fig. 2 shows regional LORETA solutions (distributed EEG sources) relative to a statistical ANOVA interaction ($F(20,1120) = 1.735$; MSe = 0.2470; $P = 0.023$) among factors Group (Responder, Non-Responder), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). As a reference, the LORETA solutions of the Nold group were also included in the diagrams of Fig. 2. Compared to Nold group, both AD sub-groups (i.e. Responders and Non-Responders) had lower amplitude of alpha sources and higher amplitude of delta sources. Here, a totally original result was that even the AD sub-group having alpha sources highest in magnitude (Non-Responders) presented lower alpha sources with reference to those of Nold subjects. This result extends previous evidence of our Consortium (Babiloni et al., 2004, 2005d).

Results of interest for the Duncan post hoc test were the regional LORETA solutions in the Responder group showing statistically significant differences ($P < 0.05$, $N = 58$) compared to the Non-Responder group. At recording I (before donepezil treatment), the frontal ($P = 0.009$), parietal ($P = 0.005$), occipital ($P = 0.014$), and temporal ($P = 0.00002$) delta sources were stronger in magnitude in the Non-Responder than in the Responder group. In addition, the occipital alpha 1 ($P = 0.000004$), temporal alpha 1 ($P = 0.00002$), and occipital alpha 2 ($P = 0.000003$) sources were enhanced in the Non-Responders. At recording II (after 1 year of donepezil treatment), the frontal ($P = 0.006$), occipital ($P = 0.00001$), and temporal ($P = 0.002$) delta sources were stronger in magnitude in the Non-Responder than in the Responder group. Furthermore, the parietal ($P = 0.015$) and occipital ($P = 0.0004$) alpha 2 sources were stronger in magnitude in Non-Responders. The numerical values of Fig. 2 are reported in Table 4 showing the mean values (±SE) of the relative LORETA current density for the Responder and Non-Responder groups in the 6 bands (delta, theta, alpha 1, alpha 2, beta 1, beta 2) and in the 5 ROIs (central, frontal, parietal, occipital, temporal) for the two mentioned EEG recordings (recording I, recording II).

Fig. 3 shows the difference of regional LORETA solutions between recordings II and I relative to a statistical ANOVA interaction ($F(20,1120) = 1.735$; MSe = 0.356; $P = 0.023$) among factors Group (Responder, Non-Responder), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). Results of interest of the Duncan post hoc test were regional LORETA solutions in the Responder group showing statistically significant differences ($P < 0.05$, $N = 58$) compared to the Non-Responder group. In both Responders and Non-Responders, the LORETA differences between the recordings II and I indicated magnitude increase of the delta and theta sources as well as magnitude decrease of the alpha sources. However, the magnitude decrement of the occipital ($P = 0.00001$) and temporal ($P = 0.041$) alpha 1 sources was significantly more marked in the Non-Responder compared to the Responder group. Numerical

Table 4
Mean values (±SE) of the relative LORETA current density for the Responder and Non-Responder groups in the 6 bands (delta, theta, alpha 1, alpha 2, beta 1, beta 2) and in the 5 ROIs (central, frontal, parietal, occipital, temporal) for the two mentioned EEG recordings (recording I, recording II)

Normalized LORETA current density (arbitrary units)						
	Delta	Theta	Alpha 1	Alpha 2	Beta 1	Beta 2
Recording I						
Responder						
Central	1.4 (±0.2)	0.85 (±0.1)	0.87 (±0.1)	0.76 (±0.1)	0.56 (±0.1)	0.41 (±0.1)
Frontal	1.68 (±0.2)	0.92 (±0.1)	0.79 (±0.1)	0.66 (±0.1)	0.59 (±0.1)	0.5 (±0.1)
Parietal	1.77 (±0.2)	1.47 (±0.2)	2.13 (±0.4)	1.62 (±0.4)	0.85 (±0.1)	0.51 (±0.1)
Occipital	1.76 (±0.1)	1.87 (±0.4)	2.48 (±0.3)	1.61 (±0.2)	0.80 (±0.1)	0.48 (±0.1)
Temporal	1.97 (±0.1)	1.71 (±0.3)	1.97 (±0.2)	1.42 (±0.2)	1.17 (±0.1)	0.9 (±0.2)
Non-Responder						
Central	1.65 (±0.2)	0.97 (±0.1)	0.92 (±0.1)	0.85 (±0.1)	0.55 (±0.1)	0.35 (±0.1)
Frontal	2.09 (±0.2)	1.06 (±0.1)	0.94 (±0.1)	0.72 (±0.1)	0.55 (±0.1)	0.46 (±0.1)
Parietal	2.19 (±0.3)	1.67 (±0.2)	2.34 (±0.3)	1.7 (±0.2)	0.8 (±0.1)	0.45 (±0.1)
Occipital	2.14 (±0.3)	2.05 (±0.2)	3.81 (±0.6)	2.3 (±0.4)	0.84 (±0.1)	0.41 (±0.1)
Temporal	2.69 (±0.3)	1.92 (±0.1)	2.59 (±0.3)	1.69 (±0.2)	1.03 (±0.1)	0.78 (±0.1)
Recording II						
Responder						
Central	1.98 (±0.3)	0.9 (±0.1)	0.61 (±0.1)	0.44 (±0.1)	0.38 (±0.1)	0.41 (±0.1)
Frontal	1.96 (±0.2)	0.88 (±0.1)	0.59 (±0.1)	0.43 (±0.1)	0.44 (±0.1)	0.57 (±0.1)
Parietal	2.19 (±0.2)	1.39 (±0.2)	1.35 (±0.3)	0.83 (±0.1)	0.52 (±0.1)	0.55 (±0.2)
Occipital	1.92 (±0.2)	1.75 (±0.4)	1.98 (±0.4)	1.05 (±0.2)	0.56 (±0.1)	0.66 (±0.2)
Temporal	2.34 (±0.2)	1.58 (±0.2)	1.5 (±0.2)	0.98 (±0.1)	0.82 (±0.1)	0.6 (±0.2)
Non-Responder						
Central	1.98 (±0.2)	1.01 (±0.1)	0.79 (±0.1)	0.66 (±0.1)	0.41 (±0.1)	0.33 (±0.1)
Frontal	2.39 (±0.2)	1.02 (±0.1)	0.72 (±0.1)	0.54 (±0.1)	0.39 (±0.1)	0.39 (±0.1)
Parietal	2.34 (±0.3)	1.41 (±0.2)	1.55 (±0.2)	1.22 (±0.3)	0.57 (±0.1)	0.4 (±0.1)
Occipital	2.49 (±0.3)	21.69 (±0.2)	2.13 (±0.3)	1.61 (±0.4)	0.67 (±0.1)	0.39 (±0.1)
Temporal	2.78 (±0.2)	1.6 (±0.1)	1.67 (±0.2)	1.5 (±0.2)	0.7 (±0.1)	0.57 (±0.1)

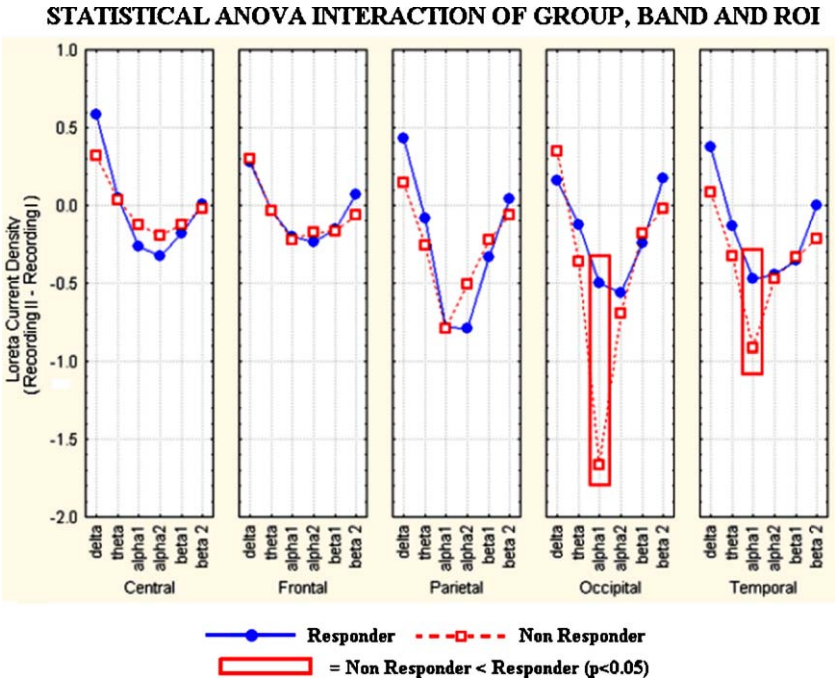

Fig. 3. Difference of regional LORETA solutions (distributed EEG sources) between recording II and I, relative to a statistical ANOVA interaction among factors Group (Non-Responder, Responder), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). This ANOVA focused on the difference of the EEG sources between recording II and I characterizing Responders with respect to Non-Responders. Legend: rectangles indicate the cortical regions and frequency bands in which LORETA solutions presented statistically different values ($P < 0.05$, Duncan post hoc test) in Non-Responder with respect to Responder subjects.

values of Fig. 3 are reported in Table 5 showing the mean values (±SE) of the difference of the relative LORETA current density between recordings II and I for the Responder and Non-Responder groups in the 6 bands (delta, theta, alpha 1, alpha 2, beta 1, beta 2) and in the 5 ROIs (central, frontal, parietal, occipital, temporal).

Correlation between regional LORETA solutions and MMSE

Before treatment (Fig. 2), the Responder group with respect to Non-Responders was characterized by different frontal, parietal, occipital, and temporal delta sources as well as different occipital alpha 1, temporal alpha 1, and occipital alpha 2 sources. The value of these sources before the treatment (recording I) was used as an input for the statistical correlation with the difference of MMSE values (II MMSE–I MMSE) in the AD group as a whole (Pearson test, $P < 0.05$). Fig. 4 shows the statistically significant results. The MMSE difference (II MMSE–I MMSE) correlated negatively with the frontal ($r = -0.31$, $P = 0.01$, $N = 58$) and temporal ($r = -0.27$, $P = 0.03$, $N = 58$) delta sources. The stronger the frontal and temporal delta sources in pre-treatment recordings, the lower was the clinical efficacy of donepezil on global cognition as measured by the MMSE change (II MMSE–I MMSE). These correlation results extend those of the ANOVA showing that pre-therapy delta sources were higher in amplitude in the group of Non-Responders

Table 5
Mean values (±SE) of the difference of the relative LORETA current density between recordings II and I for the Responder and Non-Responder groups in the 6 bands (delta, theta, alpha 1, alpha 2, beta 1, beta 2) and in the 5 ROIs (central, frontal, parietal, occipital, temporal)

Difference of normalized LORETA Current Density (arbitrary units)						
Recording II–recording I						
	Delta	Theta	Alpha 1	Alpha 2	Beta 1	Beta 2
Responder						
Central	0.58 (±0.2)	0.05 (±0.1)	−0.26 (±0.1)	−0.32 (±0.1)	−0.18 (±0.1)	−0.01 (±0.1)
Frontal	0.28 (±0.2)	−0.03 (±0.1)	−0.2 (±0.1)	−0.23 (±0.1)	−0.15 (±0.1)	−0.07 (±0.1)
Parietal	0.42 (±0.3)	−0.08 (±0.1)	−0.78 (±0.3)	−0.79 (±0.3)	−0.33 (±0.1)	−0.04 (±0.2)
Occipital	0.16 (±0.2)	−0.12 (±0.3)	−0.49 (±0.4)	−0.56 (±0.2)	−0.24 (±0.1)	−0.17 (±0.3)
Temporal	0.37 (±0.2)	−0.13 (±0.1)	−0.47 (±0.3)	−0.44 (±0.2)	−0.35 (±0.1)	−0.01 (±0.2)
Non-Responder						
Central	0.32 (±0.2)	0.04 (±0.1)	−0.12 (±0.1)	−0.19 (±0.1)	−0.12 (±0.1)	−0.02 (±0.1)
Frontal	0.29 (±0.2)	−0.03 (±0.1)	−0.21 (±0.1)	−0.17 (±0.1)	−0.16 (±0.1)	−0.06 (±0.1)
Parietal	−0.14 (±0.3)	−0.25 (±0.2)	−0.79 (±0.3)	−0.5 (±0.2)	−0.22 (±0.1)	−0.06 (±0.1)
Occipital	−0.35 (±0.3)	−0.35 (±0.3)	−1.66 (±0.6)	−0.69 (±0.3)	−0.17 (±0.1)	−0.02 (±0.1)
Temporal	−0.08 (±0.2)	−0.32 (±0.2)	−0.91 (±0.3)	−0.46 (±0.2)	−0.33 (±0.1)	−0.2 (±0.1)

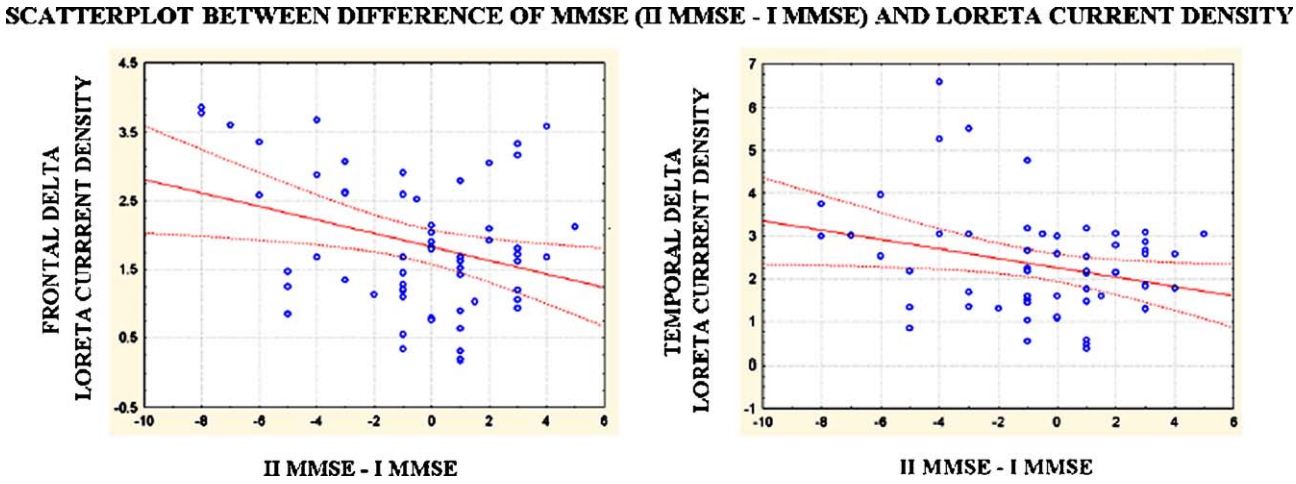

Fig. 4. Significant negative correlation ($r=-0.31$, $P=0.015$, $N=58$) between frontal delta relative density current and difference of MMSE (left side). A significant negative correlation ($r=-0.27$, $P=0.03$, $N=58$) between temporal delta relative density current and difference in MMSE scores (right side) is shown. The correlation was evaluated by Pearson test.

(loosing MMSE score at the follow-up) than in the group of Responders. Indeed, a mean difference at group level does not necessarily mean a simple correlative function at individual level. Of note, it would have been of great interest to have a finer correlative analysis of the EEG sources and the neuropsychological variables other than MMSE scores. However, the research units of the present Consortium differed in their neuropsychological protocols, thus preventing the analysis of additional neuropsychological measures in the present study.

As a control analysis, we computed correlations between the frontal and temporal delta sources before the treatment (recording I) and the difference of MMSE values (II MMSE–I MMSE) in the Responders and Non-Responders groups considered separately. The results showed a unique statistically significant negative correlations between frontal delta sources and MMSE difference in Non-Responders ($r=-0.57$; $P=0.001$, $N=28$), in line with the main results. The lack of correlation results for the temporal delta sources were possibly due to the drastic reduction of degrees of freedom and of the variation range of MMSE differences (recording II minus recording I) within each group.

Control analyses

A control ANOVA was carried out to assure that the above described LORETA source differences between Responders and Non-Responders were not due to age, education, and gender. We considered a sub-group of Responders (19 subjects) and a sub-group of Non-Responders (19 subjects) strictly overlapping in age, education, and gender distribution. Table 6 reports the means of personal and neurophysiological characteristics of the two sub-groups. The LORETA solutions were used as a dependent variable. The ANOVA factors (levels) were Group (Responder, Non-Responder; independent variable), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). There was a statistical interaction ($F(20,720)=2.27$; MSe=0.278; $P=0.0012$) among the factors Group, Recording, Band, and ROI (see Fig. 5). Results of interest for the Duncan post hoc test were the regional LORETA solutions in the Responder sub-group showing statistically significant differences ($P<0.05$), compared to the Non-Responder sub-group. At baseline Recording (before donepezil treatment), the central ($P=0.000001$), frontal ($P=0.00001$), parietal ($P=0.000001$), occipital ($P=0.000003$), and temporal ($P=0.00003$) delta sources were stronger in magnitude in the Non-Responder sub-group. In addition, the occipital alpha 1 ($P=0.000004$), temporal alpha 1 ($P=0.000006$), and occipital alpha 2 ($P=0.000002$) sources were enhanced in the Non-Responder sub-group. At recording II (after 1 year of donepezil treatment), the frontal ($P=0.00007$), occipital ($P=0.002$), and temporal ($P=0.005$) delta sources were stronger in magnitude in the Non-Responder than in the Responder sub-group. Furthermore, the occipital ($P=0.02$) alpha 2 sources were stronger in magnitude in the Non-Responders sub-group. These results fully confirmed those obtained in the whole patients sample (30 Responders and 28 Non-Responders).

To cross-validate the LORETA results on the Responder and Non-Responder groups, the analysis was directly repeated on the recorded EEG data used as input for the LORETA analyses. This was done to control for a possible effect of the source and head modeling procedures of the LORETA technique. The same frequency bands of interest of the LORETA analyses were considered, namely delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10.5 Hz), alpha 2 (10.5–13 Hz), beta 1 (13–20 Hz), and beta 2 (20–30 Hz). Analogously to the LORETA analyses, five ROIs were considered. These ROIs included respectively: (i) C3, Cz, and C4 electrodes for the central region, (ii) F3, Fz, and F4 electrodes

Table 6
Personal and neuropsychological data of interest of the Alzheimer's disease (AD) subjects, subdivided in Responders and Non-Responders

	Non-Responder	Responder
N	19	19
Age (years)	77.9 (±6.3 SD)	77.8 (±7.1 SD)
Education (years)	5.4 (±2.4 SD)	5.5 (±2.7 SD)
I MMSE	21.4 (±2.7 SD)	21.4 (±3.6 SD)
II MMSE	18 (±4.6 SD)	23.5 (±2.9 SD)
II MMSE–I MMSE	−3.4 (±2.7 SD)	+2.1 (±1.5 SD)
Gender (M/F)	3/16	3/16

The number of subjects was 19 for each group, having practically equal age, education, and gender distribution.

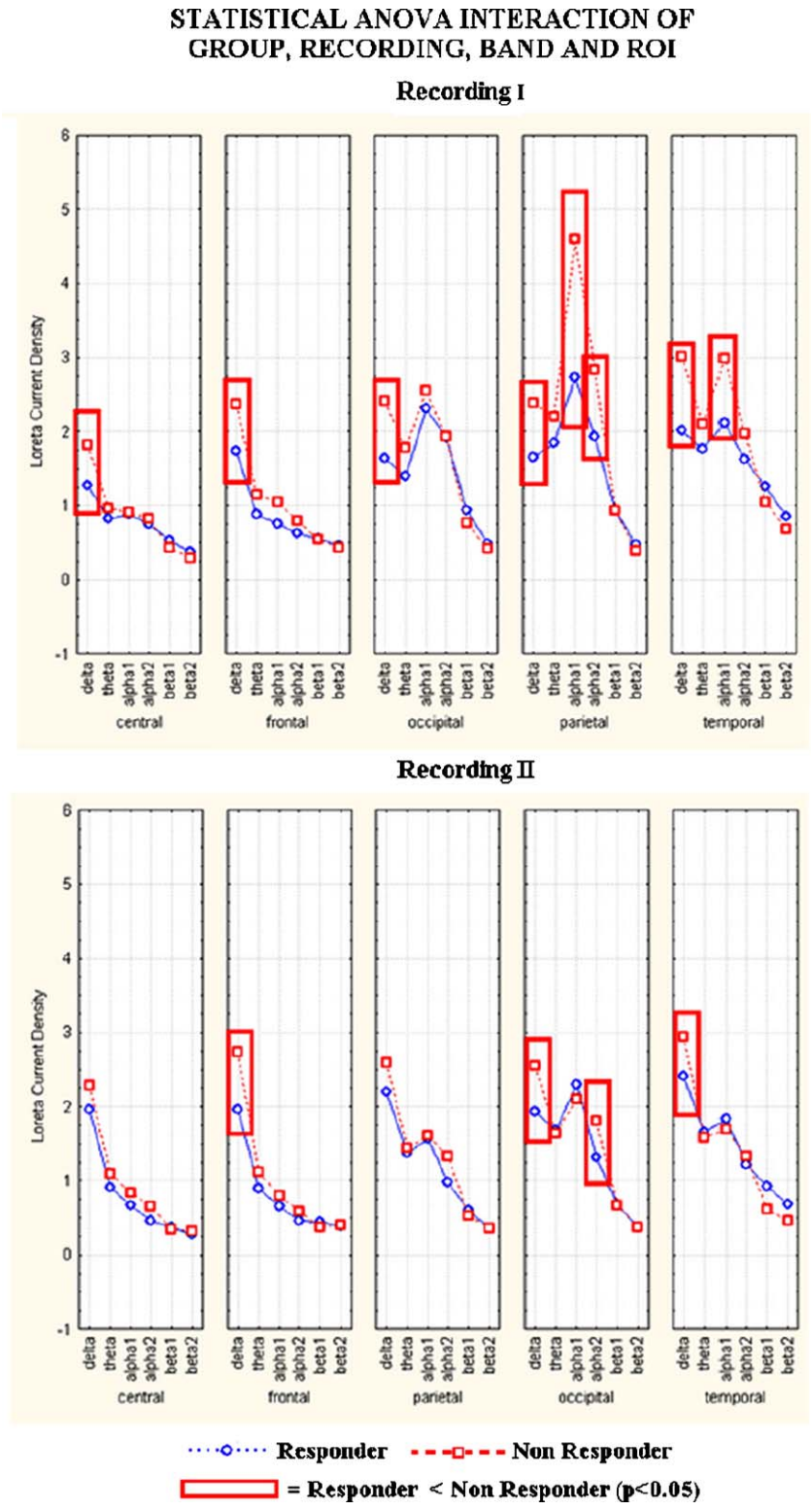

Fig. 5. Regional LORETA solutions (distributed EEG sources) relative to a statistical ANOVA interaction among factors Group (Non-Responder, Responder), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). This ANOVA design focused on the EEG sources features characterizing Responders with respect to Non-Responders before (top) and after (bottom) 1 year of donepezil treatment. The number of subjects was 19 for each group. Legend: rectangles indicate the cortical regions and frequency bands in which LORETA solutions presented statistically different values ($P < 0.05$, Duncan post hoc test) in Non-Responder with respect to Responder group.

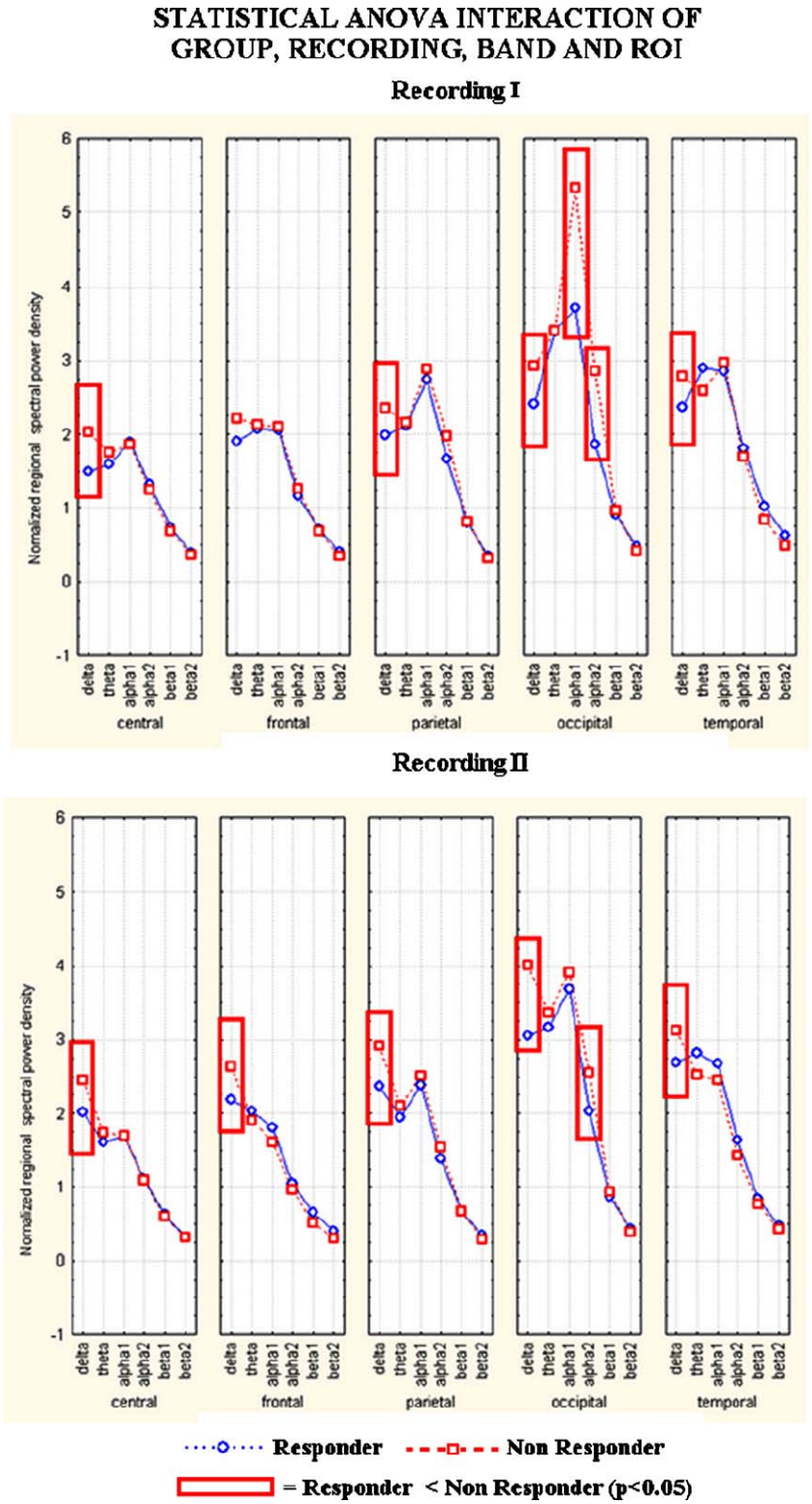

Fig. 6. Normalized regional spectral power density relative to a statistical ANOVA interaction among factors Group (Non-Responder, Responder), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). This ANOVA design focused on the EEG data used as input for the LORETA analysis to cross-validate the LORETA solutions. The EEG data (rest, eyes closed) refer to AD Responders with respect to Non-Responders. The EEG recordings were performed before (top) and after (bottom) 1 year of donepezil treatment. Of note, the number of subjects was 28 for the Non-Responder group and 30 for the Responder group. Legend: rectangles indicate the cortical regions and frequency bands in which normalized regional spectral power density presented statistically different values ($P < 0.05$, Duncan post hoc test) in Non-Responder with respect to Responder subjects.

for the frontal region, (iii) P3, Pz, and P4 electrodes for the parietal region, (iv) O1, O2 electrodes for the occipital region, (v) T3, T4, T5, and T6 for the temporal region. The same kind of normalization of the LORETA solutions was used for the EEG spectral solutions of this control analysis. The spectral power density at each electrode was normalized to the spectral power density averaged across all frequencies (0.5–45 Hz) and across all electrodes. The values of the normalized spectral power density of the electrodes belonging to the same ROI were averaged at each of the six frequency bands of interest.

The results of the control data analysis were used as inputs for an ANOVA. The values of the normalized, regional spectral power density served as dependent variables. The ANOVA factors (levels) were Group (Responder, Non-Responder; independent variable), Recording (recording I, recording II), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2), and ROI (central, frontal, parietal, occipital, temporal). Subjects' age and education were used as covariates. This ANOVA design showed a statistical interaction ($F(20,1120)=1.82$; MSe=0.308; $P=0.014$) among the factors Group, Recording, Band, and ROI (see Fig. 6). Results of interest for the Duncan post hoc testing were the normalized regional spectral power density in the Responder group showing statistically significant differences ($P<0.05$) compared to those computed in the Non-Responder group. At recording I (before donepezil treatment), the frontal ($P=0.003$), parietal ($P=0.045$), occipital ($P=0.004$), and temporal ($P=0.015$) delta sources were stronger in magnitude in the Non-Responder group. In addition, the occipital alpha 1 ($P=0.000003$) and occipital alpha 2 ($P=0.000001$) sources were enhanced in the Non-Responders. At recording II (after 1 year of donepezil treatment), the central ($P=0.017$), frontal ($P=0.009$), parietal ($P=0.001$), occipital ($P=0.00001$), and temporal ($P=0.012$) delta sources were stronger in magnitude in the Non-Responder group. Furthermore, the occipital alpha 2 sources ($P=0.003$) were stronger in magnitude in Non-Responders. On the whole, these ANOVA results fully confirmed the differences, in terms of frequency bands (delta, alpha 1, alpha 2), among Responder and Non-Responder groups based on the LORETA analysis. As expected, the effects were blurred on larger regions of the scalp when compared to those based on the LORETA source analysis.

Discussion

To our knowledge, this is the first study on pre-treatment EEG sources in patients who are Responders and Non-Responders to long-term (one year) administration of donepezil, and our results can be considered a starting point for future investigations on the neurophysiological evaluation of different sub-types of AD patients. We saw that, at baseline, that is before donepezil therapy, the posterior sources of delta, alpha 1, and alpha 2 frequencies were stronger in magnitude in patients who would not eventually respond to therapy compared to donepezil Responders (even if the two groups did not differ in global cognitive performance according to MMSE scores). Differences in delta sources were largely distributed over the cortex, whereas differences in alpha sources were localized in the temporo-occipital regions.

These findings raise two crucial questions. Why do sources of delta and alpha rhythms differ in Responders vs. Non-Responders before therapy and why are alpha sources lower in amplitude in Responders than Non-Responders? It is well known that wakeful alpha rhythms of normal amplitude characterize a healthy brain ready to process information (Steriade and Llinas, 1988; Brunia, 1999; Pfurtscheller and Lopez da Silva, 1999; Rossini et al., 1991). To formulate a tentative explanation of the present findings, generation mechanism producing EEG rhythms should be considered. During slow-wave sleep, corticofugal slow oscillations (<1 Hz) are effective in grouping thalamic-generated delta (1–4 Hz) and spindling rhythms (7–14 Hz; Steriade, 2003). In the case of brain arousal, spindles as well as high and low components of the delta rhythms are blocked by the inhibition of oscillators within, respectively, reticulo-thalamic (7–14 Hz), thalamocortical (1–4 Hz), and intracortical (<1 Hz) neuronal circuits. These rhythms are replaced by fast oscillations (beta and gamma), which are mainly induced by forebrain (nucleus basalis) cholinergic inputs to hippocampus and cortex as well as by thalamocortical projections (Steriade, 2003). In the healthy condition of awake rest, alpha (about 8–12 Hz) would dominate the EEG rhythms and delta would be low in amplitude, reminding a condition of "reciprocal inhibition". Keeping in mind this theoretical framework, it should be remarked that AD subjects not responding to the cholinergic therapy were characterized by higher power in several EEG frequencies spanning delta and alpha rhythms. The lack of selective modulation of delta and alpha rhythms may reflect some impairment of a synchronization mechanism of "reciprocal inhibition". This putative mechanism would lower delta and sustain alpha in awake normal subjects, ensuring readiness in cortical information processing within distributed cortical network.

Keeping all this in mind, at least two not mutually exclusive explanations can be used to account for the present results. First explanation is that AD patients who do not respond to donepezil are characterized by a peculiar impairment of the cholinergic basal forebrain, which alters the mechanism of "reciprocal inhibition" between the generators of the cerebral delta and alpha rhythms. In Non-Responders to cholinergic therapy, such a mechanism would fail and the generation of the delta and alpha rhythms would not be reciprocally inhibited. It should be remarked that in a parallel study we have retrospectively observed that subjects with mild cognitive impairment who converted to AD presented the same paradoxical increase of delta and alpha power when compared to subjects with mild cognitive impairment who did not convert to AD (note that the two groups were compared at a baseline period in which they had the same mean MMSE score). This explanation is in accordance with previous animal studies showing that release of acetylcholine in the cortex reduced the amplitude of delta EEG rhythms but increased the amplitude of faster rhythms (Kanai and Szerb, 1965; Celesia and Jasper, 1966). The same was true with the electrical stimulation of forebrain cholinergic neurons (Mesulam et al., 1983; Semba and Fibiger, 1989; Metherate et al., 1992) and the administration of cholinergic agonists (Cuculic et al., 1968; Metherate et al., 1992). Conversely, the intracortical application of cholinergic (muscarinic) antagonists increased the amplitude of delta and decreased that of faster EEG rhythms (Metherate et al., 1992). The same effects were obtained with experimental lesions of the basal forebrain (Stewart et al., 1984; 1984; Buzsaki et al., 1988; Ray and Jackson, 1991). A similar situation is encountered in AD, a disease deeply affecting the cholinergic basal forebrain (Rodriguez et al., 1999a,b; Dierks et al., 1993, 2000; Huang et al., 2000; Mesulam, 2004; Mesulam et al., 2004). In this framework, AD would be not characterized by a diffuse impairment of the cholinergic systems since brainstem cholinergic innervation of the thalamus was relatively spared (Mash et al., 1985; Geula and

Mesulam, 1989, 1996, 1999; Mesulam, 2004). Finally, it has been recently demonstrated that the cholinergic basal forebrain is more structurally impaired in AD patients who respond to cholinergic therapy as compared to Non-Responders (Tanaka et al., 2003).

Second explanation is that awake Non-Responders to cholinergic therapy suffer from some intrusion of spindle activity, which overlaps in frequency with the alpha rhythms (7–14 Hz) and couples the delta rhythms in slow-wave sleep (Steriade, 2003). There might be an abnormal dis-inhibition of the corticofugal slow oscillations (<1 Hz), which would trigger the thalamic-generated delta rhythms (1–4 Hz) and spindle rhythms in the awake Non-Responders to cholinergic therapy. Future studies should investigate the features of spindle EEG activity during slow-wave sleep in AD Responders and Non-Responders to donepezil therapy.

Another open question that arises from the data presented is why the differences in EEG detected at the start of the study (pre-treatment) were not associated with differences in cognitive measures in Responders and Non-Responders. Although this issue does not directly concern the fact that the EEG differentiates prior to treatment Responders from Non-Responders, it does relate to the implication that changes in the EEG are detecting cognitive changes in the patients. The relationship between EEG and clinical symptoms will deserve more attention in future investigations. At the present stage of research, it can be stated that, at least in the earlier stages of AD, similar rates of global cognitive performance according to MMSE scores might be subserved by different patterns of brain rhythmicity at delta and alpha bands. This is reasonable considering the inability of the MMSE to finely evaluate cognitive function and further stressing the need to caution against the widespread use of the MMSE as a predictor of AD progress.

Prediction of the therapy efficacy based on EEG evidence was beyond the scope of the present study since it would have required more EEG examinations over time, more neuropsychological data, as well as a more sophisticated mathematical approach. However, some of our results are of interest to this aim. We observed a negative correlation between the fronto-temporal pre-treatment delta sources and the changes of cognitive function as revealed by the difference between the baseline and follow-up MMSE values (II MMSE–I MMSE). The stronger the magnitude of the fronto-temporal delta sources before therapy, the lower the therapeutic effects of the cholinergic treatment. This was true in the group of AD patients considered as a whole. These results suggest that cholinergic treatment might fail in Non-Responders due to loss of cholinergic neurons in the frontal and temporal cortical areas, as revealed by the marked amplitude of delta sources. It can be speculated that AD patients would suffer from impairment of the cholinergic forebrain neurons combined to a neurodegenerative cascade impinging upon cholinergic hippocampal and cortical neurons. Indeed, early degeneration of the mesial temporal cortex has been previously reported in mild cognitive impairment and in AD, affecting the functional connectivity between the hippocampal formation and the cortex (Killiany et al., 1993). In AD subjects, loss of hippocampal and entorhinal neurons was correlated with the increment of cortical delta rhythms (Fernandez et al., 2003). Furthermore, delta rhythms correlated with local blood hypo-perfusion (Kwa et al., 1993; Steriade et al., 1994; Passero et al., 1995; Niedermeyer, 1997 Rodriguez et al., 1999a). In line with the present explanation, previous evidence demonstrated that cortical hypo-perfusion was more pronounced in AD Non-Responders than in Responders to cholinergic therapy (Hanyu et al., 2003a,b).

A further aim of this study was to unveil the long-term therapy effects on EEG sources in Responders to disentangle the role of cholinergic tone in sustaining cortical rhythmicity in AD subjects. Compared to Non-Responders, the Responders showed reduced deterioration of the occipital and temporal alpha sources at follow-up. These alpha rhythms may be important for higher cognitive functions such as visual attention, semantic classification, and retrieval of episodes (see Klimesch, 1999 for a review).

At this early stage of research, the mechanism underlying the long-term effects of donepezil on the cortical alpha rhythms of Responders is poorly explored. Therapy might unmask functionally silent cholinergic synaptic relays supporting feed-forward oscillatory activity at the occipito-temporal areas. Conversely, the therapy effects might be due to reactivity of less impaired neurons maintaining a sufficient level of choline acetyltransferase (Shinotoh et al., 2000). Some observations seem in keeping with this interpretation, but the data are still largely incomplete (Rodriguez et al., 2004).

The present results may have implications in the understanding of the effects of cholinergic systems on the generation of cortical EEG rhythms in normal adults. This idea is based on the assumption that long-term treatment of donepezil modulates cholinergic systems in AD Responders to the therapy. To this regard, the present results suggest that cholinergic tone may be crucial to sustain normal alpha rhythms in posterior cortex including occipital and temporal areas, in line with projections to these regions from basal forebrain cholinergic neurons of nucleus basalis (Nobili and Sannita, 1997; Oda and Nakanishi, 2000). Future investigations could evaluate the effects of preventive cholinergic therapy in elderly subjects with very mild cognitive impairment in order to probe clinical utility of this therapy at pre-clinical AD stages and to further enlighten the effects of cholinergic tone on the generation of cortical EEG rhythms in humans.

Finally, it should be stressed that this study was a retrospective collection of EEG data from several clinical research units. Unfortunately, we were unable to form a sufficient EEG database to evaluate the relationships between short- and long-term effects of donepezil therapy on cortical rhythms. Some of the clinical units received insufficient government resources for short- other than long-term EEG recordings. The present results encouraged the evaluation of these relationships in future investigations aimed at evaluating the temporal evolution of neurodegenerative effects on cortical rhythms during the cholinergic therapy. In this regard, the mechanism explaining the present results might be different from that explaining the short-term effects of therapy on the EEG rhythms. Indeed, previous studies in AD patients have shown that AChEIs mostly reduce the excess of cortical delta or theta rhythms but did not affect alpha rhythms, at least in the short run (Adler and Brassen, 2001; Adler et al., 2004; Brassen and Adler, 2003). The therapy would restore the typical abnormalities of the EEG rhythmicity around 2–12 Hz in mild AD patients (Reeves et al., 2002; Brassen and Adler, 2003). That short-term recovery contrasts with the only long-term reduction of the deterioration effects on EEG rhythms. It can be speculated a transient improvement in the short run and just a slower decline over time, in line with previous suggestions on acetylcholinesterase inhibitors (AChEIs) effects (Niedermeyer, 1997; Scott and Goa, 2000; Rodriguez et al., 2002, 2004; Hanyu et al., 2003a; Onofrj et al., 2003).

Conclusions

To our best knowledge, this is the largest EEG study on the long-term effects of cholinergic therapy in AD. A first aim of the present study was whether drugs that increase cholinergic tone can differentially modulate EEG sources in mild AD patients. Results were in favor of such a hypothesis. Before treatment, the posterior sources of delta, alpha 1, and alpha 2 frequencies were stronger in magnitude in Non-Responders compared to Responder patients. This may have implications in the prediction of the long-term effects of acetylcholinesterase inhibitors (AChEI).

A second aim of this study was to unveil the long-term therapy effects on the EEG sources of Responders to understand the in-vivo role of the cholinergic tone in sustaining cortical rhythmicity. Our results showed that, after about 1 year, a minor reduction in the occipital and temporal alpha sources characterized the Responders with respect to the Non-Responders. Therefore, a long-term increase in cholinergic tone may primarily impinge on the sources of posterior alpha rhythms, which correlate with cortical information processing sub-serving several cognitive functions. This may have implications in the understanding of the effects of cholinergic tone on the generation of normal cortical EEG rhythms. Future investigations could study the effects of preventive cholinergic therapy in elderly subjects with very mild cognitive impairment in order to probe clinical utility of this therapy at pre-clinical AD stages and to further enlighten the effects of cholinergic tone on the generation of cortical EEG rhythms in humans.

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