Neurophysiological Assessment of Alzheimer's Disease Individuals by a Single Electroencephalographic Marker

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Abstract. Here we presented a single electroencephalographic (EEG) marker for a neurophysiological assessment of Alzheimer's disease (AD) patients already diagnosed by current guidelines. The ability of the EEG marker to classify 127 AD individuals and 121 matched cognitively intact normal elderly (Nold) individuals was tested. Furthermore, its relationship to AD patients' cognitive status and structural brain integrity was examined. Low-resolution brain electromagnetic tomography (LORETA) freeware estimated cortical sources of resting state eyes-closed EEG rhythms. The EEG marker was defined as the ratio between the activity of parieto-occipital cortical sources of delta (2–4 Hz) and low-frequency alpha (8–10.5 Hz) rhythms. Results showed 77.2% of sensitivity in the recognition of the AD individuals; 65% of specificity in the recognition of the Nold individuals; and 0.75 of area under the receiver-operating characteristic curve. Compared to the AD subgroup with the EEG maker within one

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standard deviation of the Nold mean (EEG-), the AD subgroup with EEG+ showed lower global cognitive status, as revealed by Mini-Mental State Evaluation score, and more abnormal values of white-matter and cerebrospinal fluid normalized volumes, as revealed by structural magnetic resonance imaging. We posit that cognitive and functional status being equal, AD patients with EEG+ should receive special clinical attention due to a neurophysiological "frailty". EEG+ label can be also used in clinical trials (i) to form homogeneous groups of AD patients diagnosed by current guidelines and (ii) as end-point to evaluate intervention effects.

INTRODUCTION

Recent guidelines propose a diagnostic algorithm using physiopathological and topographical biomarkers of Alzheimer's disease (AD) [1], which is the most common cause of dementia in elderly subjects [2, 3]. On one hand, the physiopathological biomarkers include the measures of $A\beta_{1-42}$, total tau, and phospho-tau in cerebrospinal fluid (CSF) [23] and the maps of positron emission tomography (PET)-amyloid Pittsburg Compound B (PIB) showing the deposition of $A\beta_{1-42}$ in the brain [4–6]. These physiopathological markers would be mandatory to confirm the diagnosis of prodromal AD or dementia of AD-type in association with mild cognitive impairment (MCI) and disabling cognitive deficits [1]. On the other hand, the topographic biomarkers include the maps of FDG-PET showing AD-like brain hypometabolism [7] and the maps of magnetic resonance images (MRI) of hippocampus or cortical atrophy [8–12]. These topographic biomarkers would be especially useful to predict and track disease progression, possibly reflecting synaptic dysfunction and neural loss [1, 13]. However, none of the mentioned CSF, MRI, and PET markers allows a clear-cut diagnosis or prediction of all clinical presentations of AD. Furthermore, they cannot be serially used along years for the evaluation of AD individuals before and after pharmacological and non-pharmacological interventions. Indeed, these biomarkers are invasive (e.g., lumbar puncture for CSF sampling; the injection of radioactive tracers in PET procedures) and/or expensive (e.g., PET, MRI) for serial recordings. Therefore, there is a quest for new cost-effective, largely available, and non-invasive biomarkers of AD to be used in serial recordings and suitable for application to elderly subjects with some cognitive impairment (i.e., not requiring the subject's collaboration or prolonged states of complete immobilization).

Electroencephalographic (EEG) markers potentially fit the ideal features mentioned above [14]. The high temporal resolution of EEG signals (e.g., milliseconds) is ideal for investigating emerging features of brain physiology, namely awake brain rhythms. In the condition of resting state eyes-closed, human brain produces dominant oscillations at about 8–13 Hz, the so-called alpha rhythms [15–18]. Cognitive processes such as attention, perceptual binding, and working memory are typically related to a reduction in power of resting state alpha and beta (14–30 Hz) rhythms and to an increase in power of delta (1–4 Hz), theta (4–7 Hz), and gamma (30–70 Hz) rhythms [15–19].

Previous studies have shown that resting state eyes-closed cortical EEG rhythms typically change across physiological and pathological aging, with gradual modifications visible as a variation in EEG power (density) computed at scalp electrodes or in the activity of mathematically estimated EEG cortical sources [14, 20–28]. When compared to normal elderly (Nold) subjects, AD patients showed a power increase of topographically widespread delta and theta (4–8 Hz) rhythms and a power decrease of posterior alpha and/or beta (13–30 Hz) rhythms [22–27, 29, 30]. In addition, early stages of overt AD were typically associated to slowing down in frequency of the power peak of the resting state eyes-closed alpha rhythms, namely a decrease of the individual alpha frequency (IAF) peak [31]. In the AD patients, the mentioned abnormalities of the EEG power correlated with several relevant disease variables such as (i) markers of the amyloid cascade, as measured in the CSF [32]; (ii) resting state regional cerebral blood flow (rCBF), as measured by single photon emission computerized tomography (SPECT) or FDG-PET [26, 33]; (iii) the severity of dementia, as measured by standard clinical scales [29]; and (iv) the severity of the cognitive impairment, as indexed by Mini-Mental State Examination (MMSE) and memory score [33–37]. Finally, a marked power reduction of the posterior (i.e., especially occipital) slow-frequency alpha rhythms characterized mild AD patients when compared to patients with cerebrovascular dementia, Parkinson's disease with dementia,

and frontotemporal dementia, whereas topographically widespread theta rhythms showed higher power in cerebrovascular dementia and Parkinson's disease with dementia patients than in AD patients [24, 31, 38]. Of note, these findings are reported not to demonstrate that spectral EEG markers can be used for diagnostic purposes (they do not reflect directly $A\beta_{1-42}$ or tau). Rather, they can reveal (not diagnostic as not necessarily disease-specific) alterations of cortical neural synchronization mechanisms underlying cortical arousal related to low vigilance in groups of individuals with dementia.

While the comparison of EEG markers between groups of Nold, amnesic MCI, and AD subjects is quite useful for the understanding of the neurophysiologic correlates of AD, some important clinical applications of biomarkers stem upon the property of EEG markers to discriminate between patients and healthy controls even if they do not have diagnostic purposes. For example, EEG markers were proposed as enriching markers in the neurophysiological assessment of adults with mood and dementing disorders as well as in children with attentional and learning disabilities [39]. Furthermore, there were also characteristic patterns of EEG abnormalities in patients with alcoholic dementia [40]. Concerning the classification of Nold and AD individuals, it has been shown that spectral EEG power, or other resting state EEG features, contributed to the discrimination of Nold from mild AD individuals with 94–45% of success, from MCI to AD individuals with 92–78% of success, and the conversion of MCI to AD status with 87–60% of success [23, 41–50]. Finally, these features predicted AD in Nold subjects [51]. Despite the limited sample size, some other results were especially interesting. Surface topography of the multivariate phase synchronization discriminated between 17 Nold and 17 AD individuals with accuracy up to 94% selecting ad hoc EEG variables [50]. Furthermore, the combination of EEG markers and psychophysical indexes improved the accuracy of the classification among 32 Nold, 13 MCI, and 3 mild AD subjects up to 94% of specificity and 88% of sensitivity [52]. Finally, compressed spectral arrays estimating mean frequency analysis predicted dementia with Lewy bodies in 4 single patients originally diagnosed as AD [53].

The present study represents an important milestone of the research program of our Consortium to test EEG markers for several applications such as understanding of the neurophysiology correlates of AD, possible clinical management of patients, and definition of biomarkers to be back-translated from AD patients to preclinical mouse models of AD for drug discovery

[54]. In the framework of this research program, we have previously used a freeware called low-resolution brain electromagnetic tomography (LORETA) [55, 56] to test the hypothesis that the activity of cortical sources of delta and alpha rhythms in AD patients is related atrophy of hippocampus [57], and cerebral cortex [58], and global cognitive status as revealed by MMSE score [59]. We have also successfully tested the hypothesis that the activity of these cortical sources reflects the deterioration of cognitive status in AD patients at a follow up of about 12 months [60]. These findings confirmed that our methodological approach of cortical EEG source estimation can reveal (not diagnostic as not necessarily disease-specific) alterations of cortical neural synchronization mechanisms underlying cortical arousal related to low vigilance in AD patients.

As a further milestone and absolute novelty, this study presents and tests a single EEG marker based on posterior delta and alpha cortical sources for a neurophysiological enrichment of assessment phase in AD patients already diagnosed by current guidelines based on physiopathological biomarkers of AD and neuropsychological testing [1]. The ability of the EEG marker to classify AD individuals and matched Nold individuals was tested. Furthermore, its relation to AD patients' cognitive status and structural brain integrity was evaluated.

MATERIALS AND METHODS

Subjects

In the present study, we considered resting state eyes-closed EEG data sets of 127 AD patients and 123 Nold subjects who were recruited by qualified clinical recording units (IRCCS Fatebenefratelli Brescia, Italy; IRCCS Oasi, Troina, Italy; Service of Neurophysiopathology of the University of Genova, Genova, Italy; IRCCS "SDN", Naples, Italy; Brain Dynamics, Cognition and Complex Systems Research Center, Istanbul Kültür University, Istanbul, Turkey; Department of Neurosciences, Dokuz Eylül University, Izmir, Turkey; Unified Hospitals of Foggia, Italy). The groups were carefully matched as education, age and gender.

Table 1 summarizes the relevant demographic and clinical (MMSE) [61] data of Nold and AD participants, together with the p value of the results of the statistical comparisons between the groups. Independent *t*-test was computed to evaluate the presence or absence of statistically significant differences between the two groups (i.e., Nold and AD) for age, education, and MMSE ($p < 0.05$). Furthermore, Fisher exact

Table 1
Demographic and clinical data of the Alzheimer's disease (AD) and healthy elderly (Nold) subjects. Demographic and clinical data, together with the *p* value of the results of the statistical comparisons between Nold subjects and AD patients. MMSE, Mini-Mental State Evaluation

	Subjects (n)	Gender (M/F)	Age (years)	Education (years)	MMSE (score)
Nold	123	50/73	69.3 ($\pm$8.3 SD)	9.8 ($\pm$4.3 SD)	28.3 ($\pm$1.3 SD)
AD	127	50/77	69.3 ($\pm$8.2 SD)	9.9 ($\pm$4.8 SD)	20.4 ($\pm$4.0 SD)
Nold versus AD		$p = 0.9$	$p = 0.95$	$p = 0.75$	$p = 0.00001$

test was computed to evaluate the presence or absence of statistically significant differences between the two groups for gender ($p < 0.05$). As expected, a statistically significant difference was found for the MMSE score ($p < 0.0001$; higher MMSE score in the Nold than in the AD group). On the contrary, no statistically significant difference was found for age, gender, and education ($p > 0.05$).

The original studies including the subjects' recruitment and data collection were approved by the local Institutional ethics committee, and followed prescriptions of the Good Clinical Practice. Informed and overt consent of subjects or subjects' legal representatives was in line with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and with the standards established by the Author's Institutional Review Board.

Diagnostic criteria

Probable AD was diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR) (American Psychiatric Association. Diagnostic and statistical manual of mental disorders (IV-TR), 4th edn—text revised. Washington, DC, 2000) and the National Institute of Neurological Disorders and Stroke–Alzheimer Disease and Related Disorders (NINCDS–ADRDA) working group [62]. These accepted criteria are fulfilled in a two-step diagnostic process where there is initial identification of a dementia syndrome and then the application of criteria based on the clinical features of the AD phenotype. The DSM-IV-TR criteria require the presence of both a memory disorder and impairment in at least one additional cognitive domain, both of which interfere with social function or activities of daily living.

Most of the AD patients (121 out of 127 patients, namely more than 95%) followed a long-term treatment with standard daily doses of acetylcholinesterase inhibitors. In detail, they followed a treatment with donepezil (73 patients; 5–10 mg/die), rivastigmine (31 patients; 10 mg/die), galantamine (17 patients; 16–36 mg/die). The recruited AD patients underwent

general medical, neurological, neuropsychological (ADNI battery), and psychiatric assessments. Patients were rated with a number of standardized diagnostic and severity instruments that included MMSE [61], Clinical Dementia Rating Scale [63], Geriatric Depression Scale [64], Hachinski Ischemic Score [65], and Instrumental Activities of Daily Living scale [66]. Neuroimaging diagnostic procedures (MRI) and complete laboratory analyses were carried out to exclude other causes of progressive or reversible dementias, in order to have a clinically homogenous mild (first stage of the disease, characterized by an objective deficit on memory testing, confusion, difficulties in making decisions, in finding the right word, and other mild symptoms) to moderate AD group. Exclusion criteria included any evidence of (i) frontotemporal dementia, diagnosed according to current criteria [67], (ii) vascular dementia, diagnosed according to National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences (NINDS-AIREN) criteria [68], (iii) extra-pyramidal syndromes, (iv) reversible dementias (including pseudodementia of depression), and (v) Lewy body dementia, according to the criteria by McKeith et al. [69].

A battery of neuropsychological tests was performed to assess cognitive performance in several domains including memory (delayed recall of Rey figures, [70]; Prose Memory Test delayed recall of a story, [71]), language (1-minute verbal fluency for letters, and 1-minute verbal fluency for fruits, animals or car brands, [72]), executive function/attention (Trail Making Test part A and B, [73]), and visuo-construction abilities (copy of Rey figures [70]). Table 2 reports the mean and standard deviation (SD) values of the neuropsychological data.

The Nold subjects were recruited mostly from nonconsanguineous relatives of AD patients. All Nold subjects underwent physical and neurological examinations as well as cognitive screening (including MMSE) to exclude any type of dementia. Among them, those affected by chronic systemic illnesses (i.e., diabetes mellitus or organ failure) were excluded, as were subjects receiving psychoactive drugs. The Nold

Table 2
Mean and standard deviation (SD) values of the neuropsychological data of the AD patients

| Neuropsychological Testing in the AD Subjects		
Cognitive function	Neuropsychological test	Score
Global cognitive function	MMSE	20.4 (±3.9 SD)
Structural verbal memory	Prose Memory	3.0 (±2.8 SD)
Executive function	Trail Making Test part A	125.8 (±80.9 SD)
Executive function	Trail Making Test part B	329.0 (±285.9 SD)
Executive function	Trail Making Test B-A	225.3 (±207.3 SD)
Visuospatial and visuo-constructive abilities	Figure Rey copy	17.1 (±12.1 SD)
Visuospatial memory	Figure Rey Recall	2.5 (±3.3 SD)
Language production	Verbal fluency for letter	21.3 (±11.3 SD)
Language production	Verbal fluency for category	19.1 (±7.8 SD)
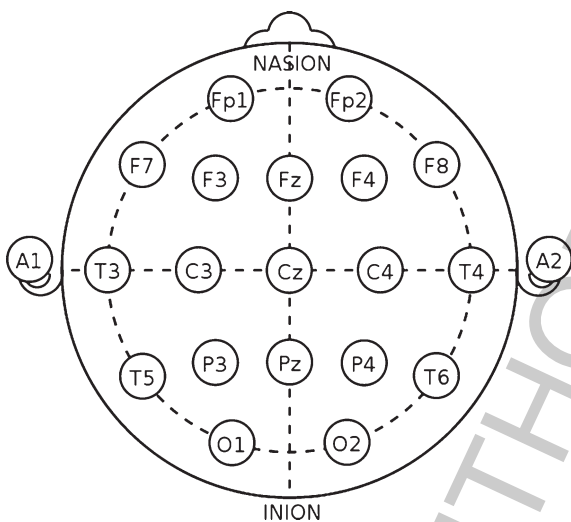
Fig. 1. Recording sites of the 19 scalp electrodes positioned according to the International 10–20 System (i.e., Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2).

subjects with history of present or previous neurological or psychiatric disease were also excluded. All Nold subjects had a Geriatric Depression Scale score lower than 14 (no depression). When they showed no sign of objective cognitive decline in the screening phase, they received neither full neuropsychological examination nor MRI scan.

EEG recordings

EEG data were recorded by the mentioned clinical units while subjects were in the condition of resting state eyes-closed for a short period (i.e., about 5 minutes). EEG recordings were performed (0.3–70 Hz bandpass) by 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; see Fig. 1). Sampling frequency ranged from 128 to 256 Hz across the mentioned clinical units,

according to their methodological facilities and standard local protocols. Analog high-bandpass was set at 0.3 Hz, while analog low-bandpass depended on the sampling frequency (e.g., 70 Hz for sampling frequency at 256 Hz). Linked earlobe reference electrode was appreciated but not mandatory, as all artifact-free EEG data were off-line re-referenced to common average. To monitor eye movements, the horizontal and vertical electro-oculogram (EOGs) were simultaneously recorded with the same recording parameters of EEG.

The EEG recordings were performed in all subjects in the late morning to minimize drowsiness. Furthermore, an operator controlled on-line the subject and the EEG traces in order to keep constant the level of vigilance.

Preliminary analysis of the EEG data

The recorded EEG data were segmented and analyzed off-line in consecutive 2 s epochs. We rejected the EEG epochs associated with operator's markers indicating drowsiness, verbal warnings, eyes opening, arm/hand movements, or other events (e.g., sweat, sway, head movements) disturbing the EEG recordings. Furthermore, the EEG epochs with ocular (e.g., rapid eye opening despite the request to maintain the eyes closed), muscular, and other types of artifacts were preliminarily identified by a computerized automatic procedure. EEG epochs with sporadic and well-shaped blinking artifacts (less than 15% of the total) were, then, corrected by an autoregressive method on the basis of the EOC activity [74]. Two independent experimenters—blind to the diagnosis at the time of the EEG analysis—manually revised the EEG epochs accepted for further analysis. They also performed a control analysis of subject's vigilance aimed at detecting EEG epochs with signs of sleep such as K complexes, sleep spindles, and slow waves.

The percentage values of the participants showing the appearance of these EEG features were 2.8% for the Nold group and 3.5% for the AD group. No statistically significant difference between the two groups was observed (Fisher exact test, $p > 0.05$ one-tailed). The EEG epochs with signs of sleep were rejected. In general, the amount of rejected EEG epochs was lower than 20% of the recorded EEG data. In detail, the percentage of artifact-free EEG epochs, after the removal of the EOG activity, was 85% for the Nold group and 81% for the AD group. A statistical procedure showed no statistically significant difference of that percentage between the two groups (Fisher exact test, $p > 0.05$ one-tailed). No EEG individual dataset accepted for final spectral analysis included less than 60 artifact-free 2-s EEG epochs.

All artifact-free EEG epochs were re-referenced to common average for further analysis.

Spectral analysis of the EEG data

A standard digital FFT-based power spectrum analysis (Welch technique, Hanning windowing function, no phase shift) was used to calculate the IAF peak, which is a frequency of special importance associated with maximum power of resting state eyes-closed EEG rhythms [15]. This standard FFT procedure was implemented by a homemade software developed under Matlab 6.5 (Mathworks Inc., Natrick, MA; Welch technique, Hanning windowing function, no phase shift, 1-Hz frequency resolution). More specifically, Hanning windowing function (i.e., 1-s windows), no overlapping windows, and no phase shift were used. We opted for two non-overlapping time windows of 1 s each, rather than for 1 time window of 2 s in any artifact-free EEG epoch lasting 2 s. This maximized the reliability of the computation of EEG mean power density and set the frequency resolution of IAF to 1 Hz (the use of time windows of 2 s would have allowed a frequency resolution of 0.5 Hz). The frequency bands of interest for the present study 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), beta 2 (20–30 Hz), and gamma (30–40 Hz), in continuity with a bulk of previous studies of our research group [24, 54, 56, 57, 59].

For a given subject, the IAF peak was defined as the frequency bin having the maximum power density averaged over all electrodes in the range from 6 to 14 Hz [15]. Mean IAF peak was 9.3 Hz (± 1.2 standard deviation, SD) in the Nold subjects and 8.6 Hz (± 1.5 SD) in the AD subjects, in line with the fact that early stages of AD are typically associated to a decrease

of the IAF peak in the EEG power density spectrum [31]. Most of AD (70.5%) and Nold (91.2%) individuals had IAF peaks within alpha 1 band (8–10.5 Hz) used to form the present EEG marker (*vide infra*). For control purposes, independent *t*-test was computed to evaluate the presence or absence of statistically significant differences between the two groups (i.e., Nold and AD) for IAF peak ($p < 0.05$). A statistically significant difference was found ($p < 0.0001$). Therefore, the IAF peak served as a covariate in subsequent statistics to minimize the possibility that differences in the IAF peak could confound the comparisons of the alpha sources between the Nold and AD groups.

Cortical source of EEG rhythms as computed by LORETA

Low resolution electromagnetic source tomography (LORETA) as provided at http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm was used for the estimation of cortical sources of scalp EEG power density [55, 75, 76]. LORETA is a source reconstruction technique belonging to a family of linear inverse solution procedures modeling 3D distributions of EEG sources [55, 75, 76]. LORETA computes 3D linear solutions (LORETA solutions) for the EEG inverse problem within a 3-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 [77]. This compartment includes 2,394 voxels (7 mm resolution), each voxel containing an equivalent current dipole fixed as position and orientation. LORETA computes relative currents for z, x, and y components of any dipole. LORETA solutions consisted of voxel current density values able to predict EEG spectral power density at scalp electrodes. Being a reference-free method of EEG analysis, in that one obtains the same LORETA source distribution for EEG data referenced to any reference electrode including common average. To enhance the topographical results, a spatial normalization was obtained by normalizing the LORETA current density at each voxel for the LORETA power density averaged across all frequencies (0.5–40 Hz) and across all 2,394 voxels of the brain volume. After the normalization, the solutions lost the original physical dimension and were represented by an arbitrary unit scale. As an advantage, this procedure reduced inter-subject variability [78, 79].

Table 3
Regions of interest (ROIs) used for the estimation of cortical sources of resting state eyes closed EEG rhythms in the present study. Any ROI is defined by some Brodmann areas of cerebral source space of low-resolution brain electromagnetic tomography (LORETA) freeware

Brodmann Areas into the 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
Limbic	31, 32, 33, 34, 35, 36

Solutions of the EEG inverse problem are underdetermined and ill conditioned when the number of spatial samples (electrodes) is lower than that of the unknown samples (current density at each voxel). In order to properly address this problem, the cortical LORETA solutions predicting scalp EEG spectral power density were regularized to estimate distributed rather than pointed EEG source patterns [55, 75, 76]. In line with the low spatial resolution of the adopted technique, we used our MATLAB software to average LORETA solutions across all voxels of a given cortical macroregion of interest (ROI) such as frontal, central, parietal, occipital, temporal, and limbic regions of the brain model (Table 3 lists the ROIs in terms of Brodmann areas as defined within the LORETA source space). For the present LORETA cortical source estimation, a frequency resolution of 0.5 Hz was used, namely the maximum frequency resolution allowed by the use of 2-s artifact free EEG epochs. In this line, the frequency bands 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), beta 2 (20–30 Hz), and gamma (30–40 Hz), in continuity with a bulk of previous studies of our research group [24, 54, 56, 57, 59].

Statistical analysis of the LORETA solutions

To compare the spatial distribution of resting state EEG sources between the Nold and the AD group, the regional normalized source solutions (i.e., source activity) were used as a dependent variable for an ANOVA design using subjects' IAF peak as covariate (STATISTICA 12; StatSoft Inc., http://www.statsoft.com). The ANOVA factors (levels) were Group (Nold, AD), ROI (frontal, central, parietal, occipital, temporal, limbic), and Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). 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 ($p < 0.05$). The planned *post-hoc* testing evaluated the prediction of regional changes in activity of the source solutions between the Nold and the AD group. Specifically, we expected: (i) a statistical 3-way interaction effect including the factors Group, ROI, and Band ($p < 0.05$); (ii) a *post-hoc* test indicating statistically significant differences of the regional normalized LORETA solutions with the pattern Nold $\neq$ AD (Duncan test, $p < 0.05$, one tailed).

Computation of the EEG marker

Based on a review of the literature (see the "Introduction") and, in particular, of our previous EEG field studies with the present methodological approach [24, 54, 56, 57, 59], the present EEG marker was defined as the ratio between the activity of parieto-occipital (LORETA) cortical sources of delta and alpha 1 rhythms. This ratio was the dependent variable in the main statistical analyses.

Accuracy of the EEG marker in the discrimination between Nold and AD individuals

As a first part of the testing, the EEG marker was used as a discriminant (not diagnostic as not necessarily disease-specific) variable for the classification between the Nold and AD individuals. The correct blind classification of this EEG marker was performed by Matlab 2010b software (Mathworks Inc., Natrick, MA, USA) for the production of receiver operating characteristic (ROC) curves [80]. The following indexes measured the classification performance of the above binary classification:

- **Sensitivity:** it measures the rate of the AD subjects who were correctly classified as AD (i.e., "true positive rate" in the signal detection theory).
- **Specificity:** it measures the rate of the Nold (control) subjects who were correctly classified as Nold (i.e., "true negative rate" in the signal detection theory).
- **Accuracy:** the mean between the sensitivity and specificity.
- **AUC:** Area under the ROC curve.

Evaluation of the relationship between the EEG marker and relevant variables of AD

Based on the values of the EEG marker (i.e., ratio between the activity of parieto-occipital delta and alpha 1 sources), we defined AD individuals with the

EEG marker "positive" (i.e., EEG+) as those having marker values equal or higher than the mean plus one standard deviation (SD) of the marker value in the Nold reference population. This EEG+ condition is expected to indicate abnormal values of the EEG marker in AD individuals as compared to the Nold group. In this line, the AD individuals with the EEG marker "negative" (i.e., EEG-) were those having marker values within the mean plus one SD of the marker value in the Nold reference population. As mentioned above, EEG+ should not be considered as a diagnostic marker as the relative abnormalities of EEG rhythms could not reflect changes of pathophysiological markers of AD according to the guidelines by Dubois and colleagues [1].

To test the hypothesis that the EEG+ condition is related to relevant disease variables in AD patients, the MMSE score was compared between the AD subgroup with EEG+ and the AD subgroup with EEG- by Student test ($p < 0.05$, one tailed; STATISTICA 12; StatSoft Inc.). The hypothesis would be confirmed by a t value indicating a statistically significant lower MMSE score in the AD subgroup with EEG+ than in the AD subgroup with EEG- ($p < 0.05$ one tailed).

In the same line, we also hypothesized that the EEG+ condition is related to more abnormal brain structure in AD patients. To test this hypothesis, cortical gray matter (GM), subcortical white matter (WM), and CSF normalized volumes were estimated in 77 out of the 127 AD patients, namely those having T1- and T2-weigthed structural MRIs available. These MRIs had been acquired following standard research settings mostly by 1.5 T scanners at the following clinical centers: IRCCS Fatebenefratelli Brescia, Italy; IRCCS Oasi, Troina, Italy; Service of Neurophysiopathology of the University of Genova, Genova, Italy; IRCCS "SDN", Naples, Italy; Brain Dynamics, Cognition and Complex Systems Research Center, Istanbul Kültür University, Istanbul, Turkey; Department of Neurosciences, Dokuz Eylül University, Izmir, Turkey; Unified Hospitals of Foggia, Italy. Some of these units collected the MRIs following the ADNI protocols available at http://www.adni-info.org (e.g., IRCCS "SDN" of Naples; United Hospital of University of Foggia; IRCCS Oasi of Troina; Service of Neurophysiopathology of the University of Genova).

The MRI scans were visually inspected to verify the absence of structural abnormalities or technical artifacts. Centralized MRI data analysis was performed by MATLAB 7.1 (MathWorks, Natick, MA) and SPM8 (Wellcome Dept. Cogn. Neurol., London; http://www.fil.ion.ucl.ac.uk/spm).

Specifically, the processing of the MRI data was as follows: the native MRI data of each patient were partitioned into GM, WM, and CSF compartments and spatially normalized to fit a standard labeled template, obtaining the transformation matrix [81]. This labeled template was based on averaged high-resolution MRIs acquired from 24 subjects, comprising anatomic channels (T1, T2, and proton density weighted), tissue channels (cerebrospinal fluid-CSF probability, gray matter-GM probability, white matter-WM probability, and tissue labels), and the LPBA40 cortical parcellation map, based on the LONI Probabilistic Brain Atlas of 40 subjects [82] and identifying 56 brain structures. Second, the inverse of the transformation matrix was used to map the 56 atlas brain structures to the partitioned GM compartment, enabling volume quantification of each structure. Thirdly, a homemade MATLAB script was used to calculate the volume of cortical GM, and of the entire (cortical and subcortical) GM, WM, and CSF compartments. The normalized volumes of cortical GM, and WM and CSF compartments were obtained dividing the volume of each compartment by the total (GM, WM and CSF) volume. Of note, the above procedure seemed to be more appropriate than voxel-based morphometry [83] for the analysis of the relationship between low-resolution (LORETA) EEG source estimates and MRI markers. Indeed, voxel-based morphometry is based on an intrinsically high-resolution voxel-by-voxel approach.

The comparison of the GM, WM, and CSF normalized volumes in the AD subgroup with EEG+ and in the AD subgroup with EEG- was performed by a two-way ANOVA using the normalized volume as a dependent variable ($p < 0.05$; STATISTICA 12, StatSoft Inc.). The ANOVA factor were Group (AD with EEG+, AD with EEG-; independent variable) and Brain volume (GM, WM, and CSF). Mauchly's test evaluated the sphericity assumption. Correction of the degrees of freedom was made with Greenhouse-Geisser procedure when appropriate. The Duncan test was used for *post-hoc* comparisons ($p < 0.05$, one tailed). The hypothesis would be confirmed by the following two statistical results: (i) a statistical ANOVA effect including the factor Group ($p < 0.05$) and (ii) a *post-hoc* test indicating statistically significant lower GM and WM normalized volumes in the AD subgroup with EEG+ than in the AD subgroup with EEG- ($p < 0.05$ one tailed) and/or a statistically significant higher CSF normalized volume in the former than in the latter subgroup ($p < 0.05$ one tailed).
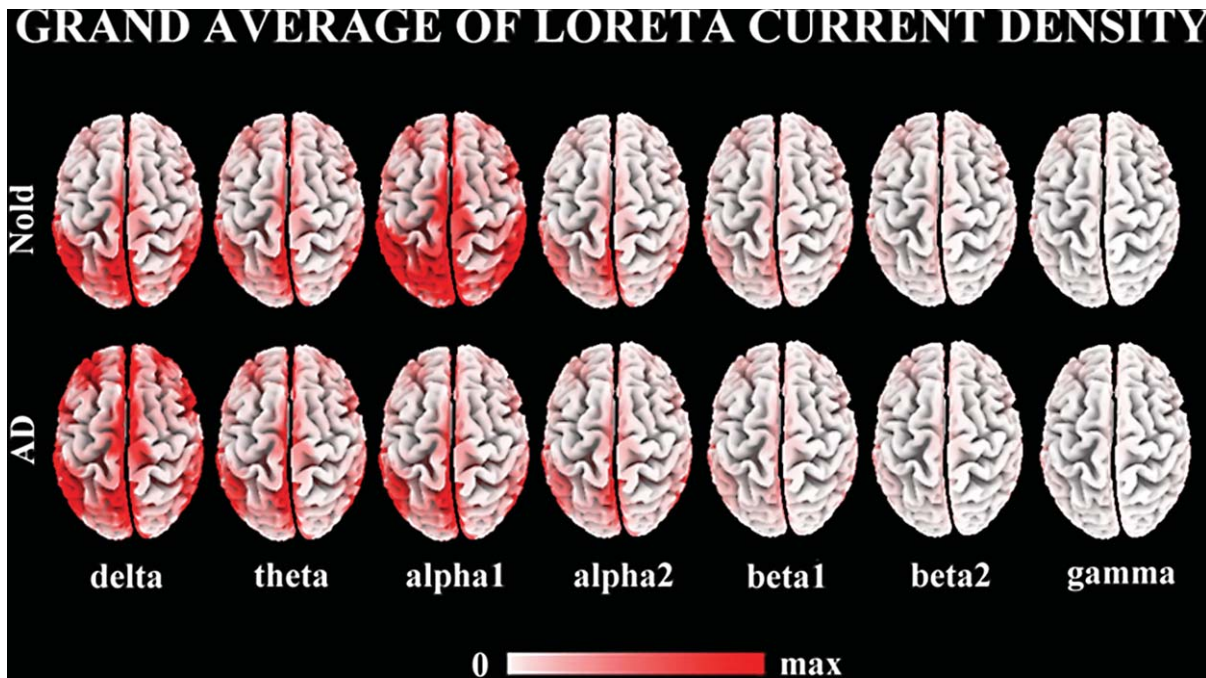
Fig. 2. Grand average of LORETA solutions (i.e., normalized relative current density at the cortical voxels or source activity) modeling the distributed EEG sources for delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma bands in the Nold and in the AD group. The left side of the maps (top view) corresponds to the left hemisphere. Legend: LORETA, low-resolution brain electromagnetic tomography. Color scale: all values of source activity were scaled based on the averaged maximum value (i.e., alpha 1 activity of occipital sources in Nold).

RESULTS

Topography of the EEG cortical sources as estimated by LORETA

For illustrative purpose, Fig. 2 maps the grand average of the LORETA solutions (i.e., relative power current density at cortical voxels or source activity), modeling the distributed EEG cortical sources for delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma bands in the Nold and in the AD group. The Nold group presented alpha 1 sources with the maximal activity distributed in the posterior regions. Delta, theta, and alpha 2 sources had moderate activity when compared to the alpha 1 sources. Finally, the beta 1, beta 2, and gamma sources were characterized by lowest activity. Noteworthy, the relative low activity of beta 2 (20–30 Hz) and gamma (30–40 Hz) cortical sources in both groups leads confirmed that the current EEG data used for source estimation were related to a condition of effective resting state and muscle relaxation. Compared to the Nold group, the AD group showed a strong activity reduction of posterior alpha sources, along with an activity increase of widespread delta sources.

Statistical analysis of the EEG cortical sources

Figure 3 shows the grand average of the activity of regional EEG cortical sources relative to a statistically significant ANOVA interaction effect $(F(30,7440) = 18.7; p < 0.0001)$ among the factors Group (Nold, AD), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic). The IAF peak was used as a covariate. Planned *post-hoc* testing (see Table 4) disclosed that the pattern Nold > AD was fitted by central, frontal, parietal, occipital, temporal, and limbic alpha 1 sources ($p < 0.05$) as well as parietal, occipital, temporal, and limbic alpha 2 sources ($p < 0.005$). On the contrary, the pattern Nold < AD was fitted by central, frontal, parietal, occipital, temporal, and limbic delta sources ($p < 0.0001$) as well as frontal, temporal, and limbic theta sources ($p < 0.005$). The present results confirm that parieto-occipital cortical sources of resting state eyes-closed delta and alpha 1 rhythms are good candidates for the computation of a valid EEG marker for a neurophysiological assessment of AD patients. We tested the value of this EEG marker in the following sections.

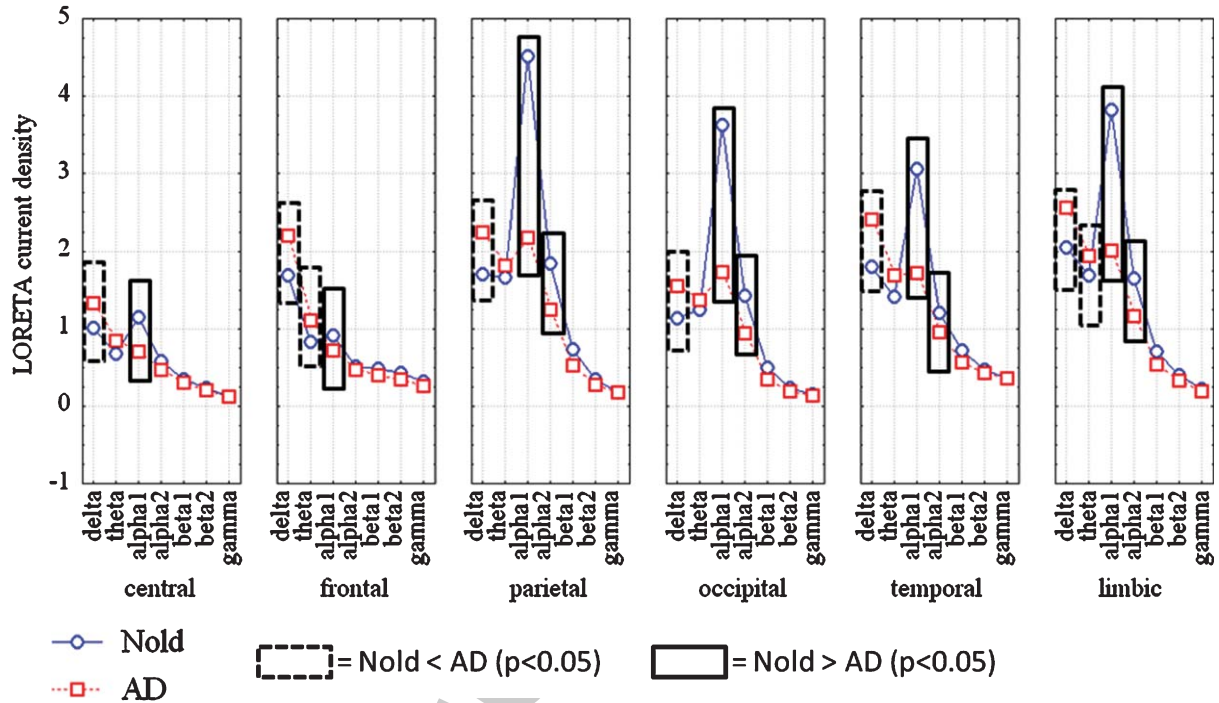## STATISTICAL ANOVA INTERACTION AMONG GROUP, BAND, AND ROI

Fig. 3. Mean values of regional normalized LORETA solutions (i.e., source activity) relative to a statistically significant ANOVA interaction effect (F(30, 7440) = 18.7; $p < 0.0001$) among the factors Group (AD, Nold), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (central, frontal, parietal, occipital, temporal, limbic). Subjects' individual alpha frequency (IAF) was used as a covariate. Legend: the rectangles indicate the cortical regions and frequency bands in which LORETA solutions (i.e., source activity) presented the statistically significant LORETA pattern of source activity Nold $\neq$ AD (Duncan test, $p < 0.05$).

Table 4
p values (Duncan *post hoc*) of the ANOVA showing a statistically significant interaction effect (F(30,7440) = 18.7; $p < 0.0001$) among the factors Group (Nold, AD), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic)

	Delta	Theta	Alpha1	Alpha2	Beta1	Beta2	Gamma
			Duncan *Post Hoc* Test Comparing EEGS Sources in the Nold and AD Subjects				
Central	$p = 0.0001$	n.s.	$p = 0.000002$	n.s.	n.s.	n.s.	n.s.
Frontal	$p = 0.000002$	$p = 0.0006$	$p = 0.01$	n.s.	n.s.	n.s.	n.s.
Parietal	$p = 0.000002$	n.s.	$p = 0.000001$	$p = 0.000001$	n.s.	n.s.	n.s.
Occipital	$p = 0.000002$	n.s.	$p = 0.000002$	$p = 0.000002$	n.s.	n.s.	n.s.
Temporal	$p = 0.000001$	$p = 0.0008$	$p = 0.000002$	$p = 0.002$	n.s.	n.s.	n.s.
Limbic	$p = 0.000004$	$p = 0.003$	$p = 0.000001$	$p = 0.000004$	n.s.	n.s.	n.s.

Accuracy of the EEG marker in the classification between Nold and AD individuals

The EEG marker served as a discriminant variable for the ROC analysis in the classification between the Nold and AD individuals. Results showed 77.2% of sensitivity in the correct recognition of the AD patients (true positive rate, expressed as a percentage), 65% of specificity in the correct recognition of the Nold subjects (true negative rate), 71.2% of accuracy (mean between the sensitivity and specificity), and 0.75 of

area under the ROC curve (see Fig. 4 and Table 5). These results indicate that the EEG marker allows a moderate classification of the individuals of the two populations (i.e., Nold, AD).

Relationship between the EEG marker and relevant variables of AD

The MMSE score as an index of global cognition was on average 21.2 (± 3.4 SD) in the AD subgroup with EEG- ($n = 86$) and 18.7 (± 4.7 SD) in the AD
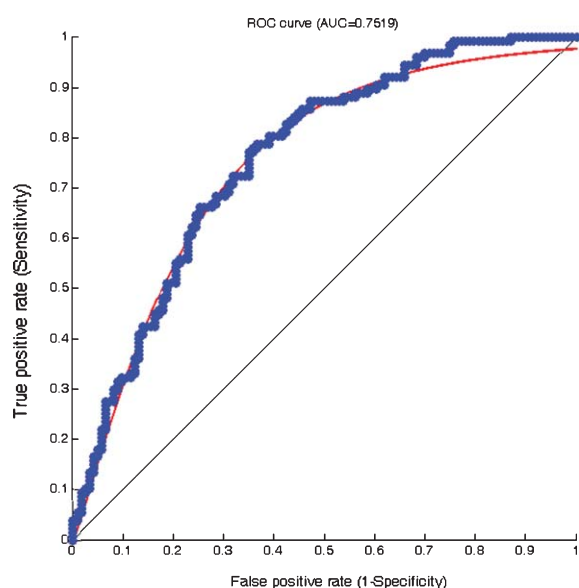
Fig. 4. ROC (receiver operating characteristic) curve illustrating the performance of the EEG marker in the classification of the Nold and AD individuals. Area under curve (AUC) was of 0.75 (moderate classification performance). True positive rate indicates the probability of the correct classification of AD datasets (sensitivity), whereas false positive rate indicates the probability of the incorrectly classification of Nold datasets (1-specificity).
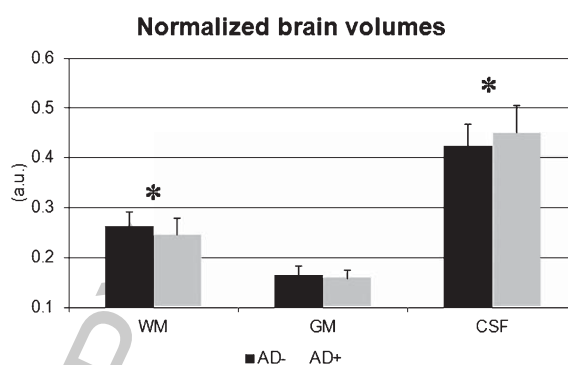

Fig. 5. Mean and SD values of cortical gray matter (GM), subcortical white-matter (WM), and cerebrospinal fluid (CSF) normalized volumes as indexes of brain structural integrity extracted by magnetic resonance imaging (MRI) in a subpopulation of 77 AD patients of the present study (those having MRI data associated to EEG recordings). The values are reported in the AD subgroup negative to the EEG marker (EEG-; $n = 36$) and in the AD subgroup positive to the EEG marker (EEG+; $n = 31$). These values refer to an ANOVA design showing a statistically significant interaction ($F(2,130) = 4.8$, $p < 0.001$) between the factors Group (AD subgroup with EEG-, AD subgroup with EEG+; independent variable) and Volume (GM, WM, CSF). Asterisks indicate the p level of the statistical differences between the two AD subgroups obtained by Duncan *post-hoc* testing.

subgroup with EEG+ ($n = 41$). Student test indicated that the difference of the MMSE score between the two AD subgroups was statistically significant ($p < 0.001$), in line with the working hypothesis.

Concerning the MRI markers of structural brain integrity, the GM, WM, and CSF normalized volumes of the AD subgroup with EEG- ($n = 36$) were on average 0.164 (± 0.02 SD), 0.263 (± 0.02 SD), and 0.423 (± 0.04 SD), respectively. Furthermore, they were 0.158 (± 0.03 SD), 0.245 (± 0.02 SD), and 0.449 (± 0.05 SD), respectively, in the AD subgroup with EEG+($n = 31$). The ANOVA design showed a statistically significant interaction ($F(2,130) = 4.8$, $p < 0.01$) between the factors Group (AD subgroup with EEG-, AD subgroup with EEG+; independent variable) and Volume (GM, WM, CSF). Duncan *post-hoc* testing indicated that the WM normalized volume was lower

in the AD subgroup with EEG+ compared to the AD subgroup with EEG- ($p < 0.05$), while the CSF normalized volume was higher in the AD subgroup with EEG+ compared to the AD subgroup with EEG- ($p < 0.001$; see Fig. 5 and Table 6). Instead, the between-groups difference in the GM volume did not reach the statistical significance ($p > 0.05$).

Control analyses

It is well known that MMSE score is an important element (e.g., among laboratory tests, patient history, neuropsychological testing, etc.) used by physicians to formulate a diagnosis of Nold or AD. Consequently, MMSE score is able to classify the present Nold and AD individuals. Keeping this fact in mind, we performed a control ROC analysis of subjects' MMSE score to produce a "ceiling" classification rate of Nold and AD individuals as a reference for the evaluation of

Table 5

Results of the classification between the Nold and AD individuals of the present study based on an EEG marker defined as the ratio of the activity between parieto-occipital cortical (LORETA) sources of delta and alpha 1 rhythms. This marker served as a discriminant variable for the analysis by receiver operating characteristic (ROC) curves

Classification Rate of the EEG marker		
	Subjects (n)	Classification as AD (%)	Classification as Nold (%)
Nold	123	35	65
AD	127	77.2	22.8

Table 6
Demographic and clinical data of the groups of AD patients (AD- and AD+). Demographic and clinical data, together with the p value of the results of the statistical comparisons between the groups of AD patients negative (EEG-) and positive (EEG+) to the EEG marker who had markers of brain integrity as revealed by magnetic resonance imaging

	Subjects (n)	Gender (M/F)	Age (years)	Education (years)	MMSE (score)
AD-	36	10/26	70.6 ($\pm$8.9 SD)	7.8 ($\pm$3.9 SD)	19.9 ($\pm$2.4 SD)
AD+	31	12/19	68.5 ($\pm$7.1 SD)	7.2 ($\pm$3.9 SD)	17.8 ($\pm$4.0 SD)
AD- versus AD+		$p = 0.4$	$p = 0.2$	$p = 0.6$	$p = 0.01$

the present EEG marker. Results showed 92.9% of sensitivity (true positive rate, expressed as a percentage), 100% of specificity (true negative rate), 96.4% of accuracy (mean between the sensitivity and specificity), and 0.98 of area under the ROC curve.

Reliability of the present results was tested by a control analysis evaluating reproducibility of the cortical estimation of resting state eyes-closed EEG rhythms in the Nold subjects. Firstly, we randomly divided all Nold subjects in two subgroups (i.e., A and B), each formed by 50% of the Nold subjects. Secondly, all artifact-free EEG epochs of any Nold subject were divided in two halves: (i) the EEG epochs of first recording half and (ii) the EEG epochs of second recording half. Thirdly, the EEG cortical sources of any Nold subject were estimated two times, one time for the EEG epochs of the first recording half and another time for the EEG epochs of the second recording half. Fourthly, we formed two populations of control Nold EEG datasets. The "first control Nold population" was formed by (i) EEG cortical sources estimated from EEG epochs of the first recording half in the subgroup A and (ii) EEG cortical sources estimated from EEG epochs of the second recording half in the subgroup B. The "second control Nold population" was formed by (i) EEG cortical sources estimated from EEG epochs of the second recording half in the subgroup A and (ii) EEG cortical sources estimated from EEG epochs of the first recording half in the subgroup B. Fifthly, we compared the spatial distribution of resting state EEG cortical sources between the "first control Nold population" and the "second control Nold population". The statistical procedure was like that used in the main analysis of the EEG cortical sources between Nold and AD populations. Specifically, the regional normalized source solutions (i.e., source activity) were used as a dependent variable for an ANOVA design (STATISTICA 12; StatSoft Inc.). The ANOVA factors (levels) were Group ("first control Nold population", "second control Nold population"), ROI (frontal, central, parietal, occipital, temporal, limbic), and Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). 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 ($p < 0.05$). Figure 6 shows the mean activity of regional EEG cortical sources in the two groups ("first control Nold population", "second control Nold population") for the ROIs (frontal, central, parietal, occipital, temporal, limbic), and for the frequency bands of interest (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). Note that the EEG cortical source profiles of the two groups were practically identical. As expected, there was no statistically significant ANOVA effect including the factor Group ($p > 0.7$), in line with a good reproducibility of the present EEG cortical source estimates.

Another test on the reliability of the present results was performed by a control analysis of correlation between the activity of parieto-occipital cortical delta or alpha 1 sources and MMSE score in all Nold and AD subjects. The control results did show a statistically significant negative correlation between the activity of parieto-occipital delta sources and MMSE score ($r = -0.26$; $p = 0.00003$; Spearman test). The higher the activity of pathological parieto-occipital delta sources, the lower the MMSE score. In the same vein, there was a statistically significant positive correlation between the activity of parieto-occipital alpha 1 sources and MMSE score ($r = 0.34$; $p = 0.00001$; Spearman test). The higher the activity of dominant parieto-occipital alpha 1 sources, the higher the MMSE score. These results pointed to a clear statistical relationship between these sources and global cognitive status in the Nold and AD subjects.

DISCUSSION

As mentioned above, the present Consortium designed an EEG research program for several neurophysiological, clinical, and preclinical applications in the AD field [54]. Most EEG markers were derived by EEG recordings in the condition of resting state eyes-closed, which reflect the fluctuation of subject's cortical arousal and vigilance. This experimental

**Mean values of regional normalized LORETA solutions
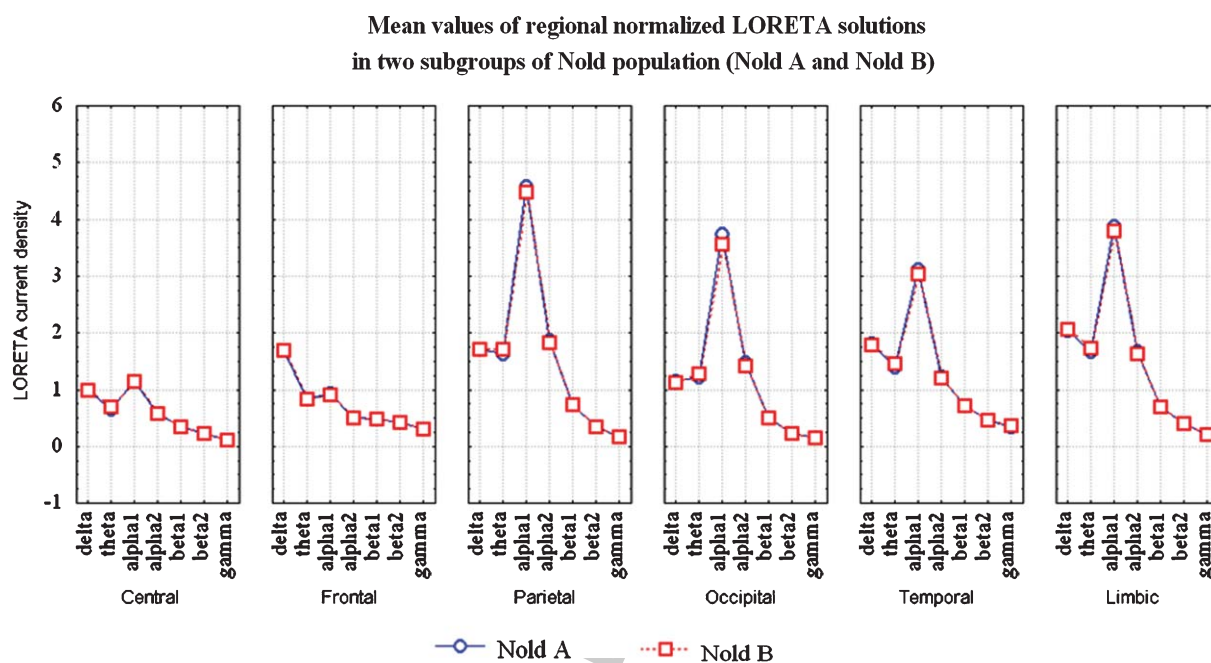in two subgroups of Nold population (Nold A and Nold B)**

Fig. 6. Mean values of regional normalized LORETA solutions (i.e., source activity) in the two subgroups of Nold population for the ROIs (frontal, central, parietal, occipital, temporal, limbic) and for the frequency bands of interest (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). Of note, the subgroups (i.e., A and B) of Nold population were formed each formed by 50% of the Nold subjects. All artifact-free EEG epochs of any Nold subject were divided in two halves: (i) the EEG epochs of first recording half and (ii) the EEG epochs of second recording half. The EEG cortical sources of any Nold subject were estimated two times, one time for the EEG epochs of the first recording half and another time for the EEG epochs of the second recording half. The "first control Nold population" (i.e., A) was formed by (i) EEG cortical sources estimated from EEG epochs of the first recording half in the subgroup A and (ii) EEG cortical sources estimated from EEG epochs of the second recording half in the subgroup B. The "second control Nold population" (i.e., B) was formed by (i) EEG cortical sources estimated from EEG epochs of the second recording half in the subgroup A and (ii) EEG cortical sources estimated from EEG epochs of the first recording half in the subgroup B.

condition has the advantage that it does not require stimulation devices or electronic systems for the control of the subject's behavior during EEG recordings and is easy to set up. For the human part of the mentioned research program, we used LORETA freeware [55] for the estimation of cortical sources of resting state eyes-closed EEG rhythms. In this study, we presented and tested a single EEG marker for a neurophysiological assessment of AD patients. The EEG marker was computed as the ratio between the activity of parieto-occipital delta and low-frequency alpha sources. Its value was tested as ability to classify single Nold and AD individuals. Furthermore, we tested its relationship to AD patients' cognitive status and structural brain integrity.

A preliminary control analysis showed that compared to the Nold group, the AD group was characterized by an activity increase of the posterior sources of delta rhythms as well as by a power decrease of the posterior sources of low-frequency alpha rhythms. Noteworthy, occipital (LORETA) source

power of these rhythms revealed the highest statistical differences between the Nold and the AD group ($p < 0.0001$), in line with previous studies using the same methodology [24, 28, 56, 57, 71, 84, 99]. The present control results confirmed the promising features of the ratio between the activity of the occipital (LORETA) sources of delta and low-frequency alpha rhythms for a simple neurophysiological assessment of AD individuals.

The first novel finding of the present study was the classification rate between the Nold and AD individuals by the new single EEG marker derived by our previous EEG studies in AD [54–60]. The results showed 77.2% of sensitivity in the correct recognition of the AD patients, 65% of specificity in the correct recognition of the Nold subjects, and 0.75 of area under the ROC curve, which corresponds to a moderate performance for a binary classifier [80, 85]. These results show an intermediate classification rate with reference to previous EEG studies reporting the following percentages of correct discrimination: 89–45% between

Nold and AD individuals, 92–78% between MCI and AD individuals, and 87–60% for the conversion of MCI subjects to AD status [23, 41–43, 45, 47–49, 86, 87]. The present moderate classification rate was reasonably due to the use of *a priori* single EEG marker for the discrimination purpose and to the intrinsic limitation of the classification procedure. Noteworthy, the ROC analysis of MMSE score provided reference sensitivity of 92.9% even if this score was used as a diagnostic variable. Furthermore, no severe AD patient (typically showing abnormal delta and alpha rhythms) was enrolled for the present study. Finally, the present AD patients were under treatment by standard long-term acetylcholinesterase inhibitors, which is expected to partially preserve EEG rhythms [59, 88–90].

The present novel EEG approach is based on a single marker and no mathematical classifier, features quite convenient for a practical use in a clinical context. The present approach parallels multivariate EEG approaches in which several EEG markers are given as an input to principal or independent component analyses. Afterwards, the outcome of these analyses is typically given as an input to trained mathematical classifiers such as support vector machines, random forest or artificial neural networks. Noteworthy, multivariate EEG approaches have previously shown classification accuracies higher than 85% in AD patients (see above literature review). In general, the univariate and the multivariate EEG approaches are not alternative and could be chosen as a function of local resources and aims. The present single EEG marker may be easily computed and used for a neurophysiological assessment in daily clinical practice, as it does not require complex mathematical platforms and reference databases. The multivariate EEG approach is more complex and may be used in memory clinics with personnel expert in the use of mathematical platforms and reference databases. Both EEG approaches could be used for research applications and clinical trials. Finally, the present single EEG marker has the advantage to be obtained by a deterministic procedure independent of rater judgment, compared to traditional visual judgment of expert neurologists of resting state EEG traces. Future studies should compare over time the single EEG marker, standard multivariate spectral EEG markers, report of traditional visual judgment of EEG activity, and MMSE score to test their clinical value in the management of AD patients.

A second novel finding of the present study was the comparison of MMSE score and MRI indexes of structural brain integrity between the AD subgroup positive (EEG+) and the AD subgroup negative

(EEG-) to the single EEG marker. The results showed that the MMSE score and WM normalized volume were lower in the AD subgroup with EEG+ compared to the AD subgroup with EEG-, while the opposite was true for the CSF normalized volume. These results were in line with the hypothesis that the EEG+ condition is related to a more abnormal global cognition and brain structure in AD patients. Therefore, the present single EEG marker was associated to relevant disease variables in the AD patients, leading support to the idea that it may enrich the clinical picture of AD individuals typically based on diagnosis performed by physiopathological biomarkers of AD and neuropsychological testing according to the current guidelines [1], disease duration, global cognitive status, and impairment in the activities of daily life. It can be speculated that the present EEG marker may better define the brain function in AD patients with the same cognitive and functional impairment and with the same cognitive reserve as indexed by education years and kind of jobs and intellectual occupations in the subject's life. The cognitive reserve is usually related to brain neural/synaptic redundancy and ability of plastic remodeling that result from the interaction of genetic, epigenetic, and environmental (i.e., prolonged periods of educational and intellectual activities) factors affecting good maturation of brain in terms of myelination of neural fibers and robust WM connections among brain regions. [91–93]. It is important in the assessment of AD patients as it reflects the adaptive processes maintaining a quasi-stable cognitive status despite ongoing physiopathological disease processes [91–93]. Keeping in mind the present results, it can be speculated that cognition, daily life abilities, and cognitive reserve status being equal [1], an AD patient with the additional feature of EEG positivity (i.e., EEG+) may reflect more neurophysiological "frailty" and less functional brain reserve than an AD patient with EEG negativity (i.e., EEG-). In this line of speculation, the AD patient with the additional feature of neurophysiological "frailty" should receive more therapeutic resources and clinical attention.

As a final methodological remark, a minority of the EEG datasets of the present study (less than 20%) was acquired by 128-Hz sampling frequency, in line with the methodological facilities and standard local protocols of some clinical recording units participating to this study (some EEG datasets of the present study were collected in the framework of local clinical routine). In this regard, it should be remarked that the use of 128-Hz sampling frequency was suboptimal for a perfect reconstruction of EEG signal up to 40 Hz

and for avoiding some distortion of low-band frequencies (the so called aliasing issue). Ideally, one should set a factor from 4 to 5 between the low-pass limit of analog bandpass filter (e.g., 40 Hz) and the EEG sampling frequency (e.g., from 160 to 200 Hz rather than 128 Hz of the present study). Setting a factor from 4 to 5 would have permitted to fully take into account the high frequency cutoffs of the roll-off analog filter and to perfectly reconstruct the highest frequency EEG contents up to 40 Hz (i.e., gamma rhythms). Aware of the above considerations, we still decided to use the EEG datasets recorded by 128-Hz sampling frequency as the present working hypothesis did focus on an EEG marker computed based on cortical sources of parieto-occipital delta and alpha rhythms, in line with previous EEG evidence of our Consortium [55–59]. In this line, cortical sources of high frequency EEG rhythms (i.e., gamma) were presented just to show as EEG power density decreased after its peak at alpha frequencies, as expected when subjects are properly relaxed during EEG recordings performed in the condition of resting state eyes closed. Of note, this condition of low vigilance should not progress to drowsiness or sleep onset during EEG recording. To mitigate this confound, the duration of the present EEG recordings was relatively short (e.g., about 5 minutes) and an experimenter monitored general subject's behavior. Furthermore, EEG epochs were carefully revised to reject those with EEG signs of drowsiness and sleep. Indeed, very few subjects showed initial EEG signs of sleep (i.e., about 3%). Finally, reliability of the present results was also tested by a control analysis of correlation between the activity of parieto-occipital cortical delta or alpha 1 sources and the MMSE score in all Nold and AD subjects. Results showed that the higher the activity of pathological parieto-occipital delta sources, the lower the MMSE score. Furthermore, the higher the activity of dominant parieto-occipital alpha 1 sources, the higher the MMSE score. These results cannot be explained by EEG sampling frequency or drowsiness and sleep during the experiments.

CONCLUSIONS

Can a single EEG marker provide a neurophysiological enrichment of the assessment phase in AD patients already diagnosed by current guidelines based on physiopathological biomarkers of AD and neuropsychological testing [1]? Based on previous evidence [56–60], we defined a single EEG marker as the ratio between the activity of parieto-occipital cortical sources of delta and low-frequency alpha rhythms. The EEG marker showed 77.2% of sensitivity in the recognition of the AD individuals, 65% of specificity in the recognition of the Nold individuals, and 0.75 of area under the ROC curve. This finding suggests that this marker can reveal (not diagnostic as not necessarily disease-specific) alterations of EEG rhythms related to low vigilance in single AD individuals. Compared to the AD subgroup with the EEG marker within one standard deviation of the Nold mean (EEG-), the AD subgroup with EEG+ showed lower global cognitive status and WM normalized volume, while the opposite was true for the CSF normalized volume. We conclude that this EEG marker may unveil a neurophysiological "frailty" in AD patients already diagnosed by current guidelines [1]. In this line, cognitive (e.g., MMSE score) and functional status being equal, AD patients with EEG+ (i.e., neurophysiological "frailty") may need special clinical attention. Future studies should test if this EEG marker is useful as an instrumental secondary endpoint to test drugs against prodromal AD or dementia due to AD and is able to monitor disease progression with a substantial added value with respect to traditional visual inspection of EEG activity and MMSE score.

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/14-3042r3).

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