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Resting State Cortical Electroencephalographic Rhythms and White Matter Vascular Lesions in Subjects with Alzheimer's Disease: An Italian Multicenter Study

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Abstract. Resting state electroencephalographic (EEG) rhythms do not deteriorate with the increase of white matter vascular lesion in amnesic mild cognitive impairment (MCI) subjects [1], although white matter is impaired along Alzheimer's disease (AD). Here we tested whether this is true even in AD subjects. Closed-eye resting state EEG data were recorded in 40 healthy elderly (Nold), 96 amnesic MCI, and 83 AD subjects. White matter vascular lesions were indexed by magnetic resonance imaging recorded in the MCI and AD subjects (about 42% of cases following ADNI standards). The MCI subjects were divided into two sub-groups based on the median of the white matter lesion, namely MCI+ (people with highest vascular load; $n = 48$) and MCI− (people with lowest vascular load; $n = 48$). The same was true for the AD subjects (AD+, $n = 42$; AD−, $n = 41$). EEG rhythms of interest were delta (2–4 Hz), theta (4–8 Hz), alpha1 (8–10.5 Hz), alpha2 (10.5–13 Hz), beta1 (13–20 Hz), beta2 (20–30 Hz), and gamma (30–40 Hz). LORETA software estimated cortical EEG sources. When compared to Nold group, MCI and AD groups showed well known abnormalities of delta and alpha sources. Furthermore, amplitude of occipital, temporal, and limbic alpha 1 sources were higher in MCI+ than MCI− group. As a novelty, amplitude of occipital delta sources was lower in AD+ than AD− group. Furthermore, central, parietal, occipital, temporal, and limbic alpha sources were higher in amplitude in AD+ than AD− group. Amplitude of these sources was correlated to global cognitive status (i.e., Mini Mental State Evaluation score). These results suggest that in amnesic MCI and AD subjects, resting state posterior delta and alpha EEG rhythms do not deteriorate with the increase of white-matter vascular lesion. These rhythms might be more sensitive to AD neurodegenerative processes and cognitive status rather than to concomitant lesions to white matter.

Keywords: Alzheimer's disease, Alzheimer's disease neuroimaging initiative, amnesic mild c cognitive impairment, electroencephalographic rhythms, low resolution brain electromagnetic tomography, magnetic resonance imaging, resting state, white matter vascular lesion

INTRODUCTION

Previous studies in Alzheimer's disease (AD) and amnesic mild cognitive impairment (MCI) subjects have shown that resting state closed-eye EEG rhythms may be promising markers of disease when evaluated by quantitative methods. When compared to normal elderly (Nold) subjects, AD patients have been characterized by high power of delta (0–4 Hz) and theta (4–7 Hz) rhythms, and low power of posterior alpha (8–12 Hz) and/or beta (13–30 Hz) rhythms [2–8]. In line with the "transition" hypothesis, amnesic MCI subjects have displayed increased theta power [9–11] as well as decreased alpha power [4, 8, 10–15]. Furthermore, power of resting state alpha rhythms in amnesic MCI subjects has been found to be intermediate with respect to that of Nold and AD subjects [6, 12, 14].

A bulk of previous evidence indicates that power of resting state eyes closed EEG rhythms reflect neurodegenerative processes in amnesic MCI and AD subjects [4, 8, 9, 12, 14, 16]. First, in MCI and AD subjects, abnormalities of EEG rhythms were associated to typical signs of neurodegeneration such as hippocampal atrophy [17] and impairment of the cholinergic tracts from basal forebrain to cerebral cortex [17, 18]. Second, these abnormalities were also associated to altered regional cerebral blood flow/metabolism and to impaired global cognitive function in MCI or AD subjects [1, 6, 14, 19–21]. Third, decrement of posterior alpha power showed peculiar features in AD subjects when compared to cerebrovascular dementia subjects with similar cognitive impairment [7]. Fourth, posterior alpha power was relatively preserved in amnesic MCI subjects in whom cognitive decline was mainly explained by white-matter vascular lesion, thus suggesting that these rhythms are less affected by diffuse white matter vascular lesions than parallel neurodegenerative processes [1, 23]. This hypothesis is in line with recent evidence showing that there were fewer neurodegenerative lesions in AD patients with vascular lesions than in those without vascular lesions, suggesting that neurodegenerative and cerebrovascular lesions act as additive/synergistic causes of AD [24–26]. On the other hand, several field studies have reported some interactions between AD and cerebrovascular function. Clinical and cognitive status of AD patients was in part explained by amyloid angiopathy of small vessels [27]. Furthermore, AD patients carrying ApoE4 allele as a genetic risk of AD presented an increment of intima-media thickness values with respect to non-carriers and cerebrovascular dementia patients [28]. Finally, evolution of cognitive function in AD was unfavorable as a function of impaired cerebral vasomotor reactivity [29]. Keeping in mind these data and considerations, current evidence suggests that cerebrovascular dysfunction precedes and accompanies cognitive dysfunction and AD neurodegeneration, although its impact on the abnormalities of resting state

Table 1
Demographic and clinical data of healthy elderly (Nold), mild cognitive impairment (MCI), and mild Alzheimer's disease (AD) subjects

	Subjects (n)	Gender (M/F)	Age (years)	MMSE	IAF (Hz)	IADL	CDR
Nold	40	22/18	72.1 ± (1.0)	27.7 ± (0.2 SE)	9.3 ± (0.2)	–	–
MCI	96	32/64	71.4 ± (1.0)	25.9 ± (0.3 SE)	9.5±(0.1)	2.3 (±0.1 SE)	0.3 (±0.06 SE)
AD	83	25/58	69.8 ± (0.8)	19.6 ± (0.5 SE)	8.8 ± (0.2)	4.5 (±0.3 SE)	1.0 (±0.1 SE)

EEG rhythms in AD might be negligible. To address to this issue, the present study tested the hypothesis that in both amnesic MCI and AD subjects, posterior resting state EEG rhythms do not deteriorate with the increase of white-matter vascular lesion, according to the idea that these rhythms are less affected by such vascular than neurodegenerative processes.

METHODS

Subjects

In this study, 96 amnesic MCI subjects and 83 AD patients were recruited. Furthermore, 40 cognitively intact elderly (Nold) subjects were selected as a control group. The Nold subjects globally matched the personal variables of the MCI and AD subjects. Table 1 reports demographic and clinical data of the AD, amnesic MCI, and Nold groups.

The study was approved by the local Institutional ethics committee, and follows prescriptions of the Good Clinical Practice (GCP); informed and overt consent of subjects or subjects' legal representatives, in line with 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

The present inclusion and exclusion criteria for amnesic MCI subjects were based on international standards [30–39]. Summarizing, the inclusion criteria were as follows: (i) objective memory impairment on ADNI neuropsychological evaluation probing cognitive performance in the domains of memory, language, executive function/attention, etc; (ii) normal activities of daily living as documented by the history and evidence of independent living; and (iii) clinical dementia rating score of 0.5.

The exclusion criteria included: (i) mild dementia of the AD type, as diagnosed by standard protocols including NINCDS-ADRDA [40] and DSM-IV; (ii) evidence (including magnetic resonance imaging – MRI – procedures) of concomitant cerebral impairment such as frontotemporal degeneration, cerebrovascular disease with large vascular lacunar lesions in gray or white matter, and reversible cognitive impairment (including pseudo-depressive dementia); (iii) marked fluctuations in cognitive performance compatible with Lewy body dementia and/or features of mixed cognitive impairment including cerebrovascular disease (particular attention was devoted to this point given the working hypothesis focused on cognitive stability in MCI subjects); (iv) evidence of concomitant extra-pyramidal symptoms; (v) clinical and indirect evidence of depression as revealed by the Geriatric Depression Scale (GDS; [41]) scores >14; (vi) other psychiatric diseases, epilepsy, drug addiction, alcohol dependence (as revealed by a psychiatric interview) and use of psychoactive drugs including acetylcholinesterase inhibitors or other drugs enhancing brain cognitive functions; and (vii) current or previous uncontrolled or complicated systemic diseases (including diabetes mellitus) or traumatic brain injuries.

Probable AD was diagnosed according to NINCDS-ADRDA [40] and DSM IV criteria. The recruited AD patients underwent general medical, neurological, neuropsychological, and psychiatric assessments. Patients were rated with a number of standardized diagnostic and severity instruments that included Mini Mental State Evaluation (MMSE; [42]), Clinical Dementia Rating Scale (CDR; [43]), GDS [41], Hachinski Ischemic Score (HIS, [44]), and Instrumental Activities of Daily Living scale (IADL, [45]). Neuroimaging diagnostic procedures (MRI) and complete laboratory analyses were carried out to exclude other causes of progressive or reversible dementias. Exclusion criteria included any evidence of (i) frontotemporal dementia, diagnosed according to current criteria [46], (ii) MRI of cerebrovascular disease with large vascular lacunar lesions in gray or white matter (iii) vascular dementia, diagnosed according to NINDS-AIREN criteria [47], (iv) extra-pyramidal syndromes, (iv) reversible dementias (including pseudodementia of depression); and (v) Lewy body dementia, according to the criteria by [48].

The present Nold subjects were recruited mostly from non-consanguineous patients' relatives. All Nold subjects underwent physical and neurological examinations as well as cognitive screening (including MMSE and GDS). Subjects affected by chronic systemic illnesses, those receiving psychoactive drugs, or with a history of neurological or psychiatric disease were excluded. All Nold subjects had a GDS score lower than 14 (no depression).

Magnetic Resonance Imaging (MRI)

Three-D proton density (PD), T1- and T2-weighted volumetric MRIs were recorded by the clinical units of the present Italian multi-centric study (University of Foggia-Ospedali Riuniti di Foggia; San Raffaele Cassino; Isola Tiberina Fatebenefratelli Hospital, Rome; IRCCS Fatebenefratelli Brescia; IRCCS Centro Neurolesi, Messina; Azienda Ospedaliera Sant'Andrea University of Rome "Sapienza"; University of Naples "Federico II"; Second University of Naples; University "Campus Biomedico" Rome; IRCCS and Fondazione SDN Naples; IRCCS Oasi, Troina). Some of these units (IRCCS Centro Neurolesi "Bonino-Pulejo", Messina; Azienda Ospedaliera Sant'Andrea University of Rome "Sapienza"; University of Naples "Federico II"; Second University of Naples; University "Campus Biomedico" Rome; IRCCS and Fondazione SDN Naples; IRCCS Oasi, Troina) collected the MRIs following the ADNI protocol (http://www.adni-info.org/). In total, about 42% of the whole dataset was collected according to the ADNI project.

Analysis of the 3-D PD, T1-, and T2-weighted volumetric MRIs was centralized at University of Rome "Sapienza". The MRIs were visually inspected to verify the absence of structural abnormalities or technical artifacts. Afterwards, they were given as an input to Expectation-Maximization Segmentation (EMS) software, which is an SPM99 tool (Wellcome Dept. Cogn. Neurol., London; http://www.fil.ion.ucl.ac.uk/spm) running under MATLAB 7.0 (MathWorks, Natick, MA). On the whole, the EMS tool performs (i) an automated, atlas-based classification of brain tissue from 3-D PD, T1- and T2-weighted volumetric MRIs, (ii) builds a stochastic individual model of "normal" tissue intensity at voxel level on the basis of all MRIs, (iii) detects voxels with "vascular lesion" by the computation of the Mahalanobis distance. In detail, individual MRIs were corrected for field inhomogeneities and coregistered each other. The coregistered MRIs were normalized to the SPM99 T1 template, which allowed the classification of the voxels into three compartments including gray matter, white matter or cerebral-spinal fluid. Afterwards, the EMS tool estimated the parameters of a stochastic model of tissue intensity for "normal" brain MRIs in each individual normalized dataset. Tissue intensities for the "normal" brain model were represented with a 3-classes (i.e., gray matter, white matter, and cerebral-spinal fluid) finite multivariate Gaussian mixture. All MRI sequences (i.e., 3-D PD, T1-, and T2-weighted) were used to create a multidimensional feature space, in order to benefit of the specific inherent information of each sequence. These sequences were iteratively classified into a small number of Gaussian distributions. During this iterative process, the EMS tool rejected voxels that exceed a predefined Mahalanobis distance to each of the Gaussians, and updated the model parameters only based on non-rejected voxels [49]. Vascular lesion of white matter was defined as the amount of voxels classified as affected by vascular lesion and rejected from the stochastic model of "normal brain", according to the mentioned Mahalanobis distance. In this framework, the use of Markov random fields (MRF) discouraged a voxel to be classified as brain lesion in the absence of neighboring white matter.

Of note, the EMS tool implements an automated procedure that requires only the Mahalanobis distance threshold parameter (k) to be computed on the basis of a variable defined by the experiment; namely, the parameter k determined the significance level at which voxels are considered as model outliers. An appropriate k value had to be chosen in advance by means of an experimentally tuned procedure, because of the choice of k significantly affects the quality of the brain lesion segmentation [49, 50]. The optimal value of the parameter k was identified on 36 MRI individual datasets. The MRI segmentations were obtained with the automatic tool varying the values of k from 3.0 to 5.0 with steps of 0.5. The results were correlated to those of the MRI segmentation performed by our expert neuroradiologists. The highest correlation was obtained with parameter k equal to 4.0 (Pearson $r = 0.7$; $p = 0.001$), which is exactly the threshold value suggested by the researcher who developed the EMS tool for the detection of vascular lesions in multiple sclerosis patients (http://www.medicalimagecomputing.com).

Based on the above procedure for the estimation of white-matter vascular lesions, the MCI subjects were divided into two sub-groups. The median of the white matter lesion was used as the criterion of the definition of MCI people with highest vascular load or MCI + ($\geq$4960 voxels; mean of 7479 voxels $\pm$ 483 standard error, SE; $n = 48$) and of MCI people with

Table 2
Demographic and clinical data of the following sub-groups: AD with low degree of white-matter lesion (AD−, normalized white-matter vascular lesions <3430); AD with high degree of white-matter lesion (AD+, normalized white-matter vascular lesions ≥3430); MCI with low degree of white-matter lesion (MCI−, normalized white-matter vascular lesions <4960) and MCI with high degree of white-matter lesion (MCI+, normalized white-matter vascular lesions ≥4960)

	Subjects (n)	Gender (M/F)	Age (years)	MMSE	IAF (Hz)	White matter vascular lesion (voxels)
MCI+	48	16/32	69.6 ± (1.2 SE)	26.5 ± (0.4 SE)	9.6 ± (0.2 SE)	7479 ± (483 SE)
MCI−	48	16/32	70.1 ± (1.0 SE)	25.4 ± (0.4 SE)	9.3 ± (0.2 SE)	3332 ± (179 SE)
AD+	42	13/29	72.2 ± (1.2 SE)	21.0 ± (0.6 SE)	8.9 ± (0.2 SE)	8744 ± (1295 SE)
AD−	41	12/29	70.6 ± (1.5 SE)	18.4 ± (0.8 SE)	8.7 ± (0.3 SE)	2298 ± (107 SE)

lowest vascular load or MCI− (<4960 voxels; mean of 3332 voxels ± 179 SE; $n = 48$). The same criterion based on the median of the white matter lesion was used to divide AD subjects into the sub-groups of AD+ (≥3430 voxels; mean of 8744 voxels ± 1295 SE; $n = 42$) and AD− (<3430 voxels; mean of 2298 voxels ± 107 SE; $n = 41$). Table 2 reports demographic and clinical data of the AD−, AD+, MCI−, and MCI+ sub-groups.

EEG recordings

Resting state eyes closed EEG data were recorded (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) and referenced to linked earlobes or cephalic reference. To monitor eye movements, the horizontal and vertical electrooculogram (0.3– 70 Hz bandpass) was simultaneously recorded. All data were digitized in continuous recording mode (about 5 min of EEG; 128–512 Hz sampling rate, the sampling rate being fixed in each recording research unit of this multi-centric study). In all subjects, EEG recordings were performed in the late morning. In order to keep constant the level of vigilance, an operator controlled on-line the subject and the EEG traces, verbally alerting the subject any time there were signs of behavioral and/or EEG drowsiness.

Preliminary analysis of the EEG data

The recorded EEG data were segmented and analyzed off-line in consecutive 2 s epochs. The EEG epochs with ocular, muscular, and other types of artifacts were preliminarily identified by a computerized automatic procedure. EEG epochs with sporadic blinking artifacts (less than 15% of the total) were then corrected by an autoregressive method [51]. Two independent experimenters – blind to the diagnosis at the time of the EEG analysis – manually confirmed the EEG segments accepted for further analysis. Finally, we re-referenced artifact free EEG data to common average for further analysis.

Spectral analysis of the EEG data

The digital FFT-based power spectrum analysis (Welch technique, Hanning windowing function, no phase shift) was evaluated in order to calculate the individual alpha frequency (IAF) peak, defined as the frequency associated to the strongest EEG power at the extended alpha range of 6–13 Hz [53]. Mean IAF peak was 9.3 Hz (±0.2 SE) in the Nold subjects, 9.5 Hz (±0.1 SE) in the MCI subjects, and 8.8 Hz (±0.2 SE) in the AD subjects. No statistically significant ANOVA differences were found ($p > 0.05$). However, the IAF peak was used as a covariate (together with age, gender, recording unit site, and use or not of the ADNI protocol in the statistics analyses. Indeed, the IAF is a frequency of special importance, since it is associated with maximum power of resting eyes-closed EEG rhythms [52]. The above procedure minimized the possibility that small differences in the IAF peak could confound the comparisons among the Nold, MCI, and AD groups. The standard 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 reference previous studies on the cortical sources of resting EEG rhythms in pathological aging [8, 14, 53–56]. Choice of the fixed EEG bands did not account for IAF peak. However, this should not affect the results, since more than 90% of the subjects had the IAF peaks within the alpha 1 band (8–10.5 Hz) and the IAF was used as a covariate in the statistical analysis.

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 EEG rhythms [57–59]. LORETA is a functional imaging technique belonging to a family of linear inverse solution procedures [60] modeling 3D distributions of EEG sources [59]. With respect to the dipole modeling of cortical sources, no a priori decision of the dipole position is required by LORETA procedure. LORETA belongs to the family of linear inverse algorithms like minimum norm solution, weighted minimum norm solution or weighted resolution optimization [58, 61, 62], and has been successfully used in recent EEG studies on pathological brain aging using the same experimental set up (electrode montage, sample frequency, etc.) of the present study [3, 7, 14, 53–56].

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 [63]. This compartment includes 2394 voxels (7 mm resolution), each voxel containing an equivalent current dipole. Of note, EEG electrode positions were not co-registered to individual brain source models; unfortunately, the official LORETA package did not include software to do so and we could not obtain the digitalization of the electrode position from our clinical units. LORETA can be used from EEG data recorded by low spatial sampling of 10–20 system (19 electrodes) when cortical sources are estimated from resting state eyes-closed EEG rhythms [1, 7, 14, 17, 23, 53–56, 64–71]. Indeed, resting state eyes-closed EEG rhythms are generated by coherent synchronous neural activity of large cortical areas (i.e., the summed activity of a large number of pyramidal neuron assemblies). As a result, these rhythms are characterized by low-spatial frequency content that can be properly sampled by the 19 scalp electrodes placed according to 10–20 system [72].

LORETA solutions consisted of voxel z-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. A normalization of the data was obtained by normalizing the LORETA current density at each voxel with the power density averaged across all frequencies (0.5–45 Hz) and across all 2394 voxels of the brain volume. After the normalization, the solutions lost the original physical dimension and were represented by an arbitrary unit scale. This procedure reduced inter-subjects variability and was used in previous EEG studies [7, 14, 53–56]. The general procedure fitted the LORETA solutions in a Gaussian distribution and reduced inter-subject variability [73, 74]. Other methods of normalization using the principal component analysis are effective for estimating the subjective global factor scale of the EEG data [75]. 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 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 punctual EEG source patterns [57–59]. In line with the low spatial resolution of the adopted technique, we used our MATLAB software to collapse all voxels of LORETA solutions within each of the cortical macroregions of interest (ROIs) such frontal, central, parietal, occipital, temporal, and limbic regions of the brain model. The belonging of a LORETA voxel to a Brodmann area was defined by original LORETA package. Table 3 lists the Brodmann areas (BAs) represented into each ROI.

A main advantage of the regional analysis of LORETA solutions, using an explicit source model coregistered into Talairach space, was that our modeling could disentangle rhythms of contiguous cortical areas (namely those from the occipital source were disentangled with respect to those of the contiguous parietal and temporal sources, etc).

Statistical analysis of the LORETA solutions

Statistical analysis aimed at evaluating two main working hypotheses. These hypotheses were the following: (1) LORETA solutions of resting state cortical

Table 3
Brodmann areas included in the cortical regions of interest (ROIs) of the present study. LORETA solutions were collapsed in frontal, central, parietal, occipital, temporal, and limbic ROIs

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
Limbic	31, 32, 33, 34, 35, 36

EEG rhythms show difference in amplitude among the Nold, MCI, and AD subjects; (2) LORETA solutions point to difference in amplitude between AD+ and AD− groups as well as between MCI+ and MCI− groups. The LORETA solutions showing such significant differences are correlated to the cognitive status as revealed by MMSE score.

To test the first working hypothesis, the LORETA solutions values were used as a dependent variable for an ANOVA design using subjects' age, gender, MMSE, IAF peak, and recording unit site as covariates. The ANOVA factors (levels) were Group (Nold, MCI, AD), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic). 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 ($p < 0.05$). Specifically, the working hypothesis would be confirmed by a statistical ANOVA effect including the factor Group ($p < 0.05$), and planned post-hoc testing showing differences in line with the pattern Nold $\neq$ MCI $\neq$ AD ($p < 0.05$).

To test the second working hypothesis, the LORETA solutions values were used as a dependent variable for an ANOVA design using subjects' age, gender, MMSE, IAF peak, and recording unit site as covariates. The ANOVA factors (levels) were Group (MCI+, MCI−, AD+, AD−), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic). 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 working hypothesis would be confirmed by a statistical ANOVA effect including the factor Group ($p < 0.05$), and planned post-hoc testing showing differences between AD+ and AD− groups as well as between MCI+ and MCI− groups. Finally, EEG sources showing these statistically significant differences as a function of the white matter vascular lesions were correlated to MMSE score in the continuum of the MCI and AD subjects as a whole group (Pearson test, $p < 0.05$).

Novelty of the present study

This study is a part of larger scientific program on EEG markers of AD, yet it is well framed and distinct from the previous studies of the Authors [7, 14, 53–56]. Specifically, this is our first study examining the relationships between resting state EEG sources and white matter vascular lesions in AD. To address this issue, we performed an unedited analysis of white matter vascular lesions by EMS-SPM software in 83 AD patients and 96 amnesic MCI subjects, for the comparison of EEG sources between sub-groups of these subjects with a different degree of white matter vascular lesion. Among these subjects, no AD patient and 64 amnesic MCI subjects had been previously used in the reference investigations evaluating white matter vascular lesions by Wahlund visual rating scale [1, 23]. The results of the present analysis are absolutely original (i.e., never published before).

RESULTS

Figure 1 shows the grand average of regional normalized LORETA solutions (i.e., relative power current density averaged with each ROI)) relative to an ANOVA interaction ($F(60,6480) = 11.91$; $p < 0.0001$) among the factors Group (Nold, MCI, AD), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic). Planned post-hoc testing indicated that occipital alpha2 sources as well as parietal, occipital, temporal, and limbic alpha 1 sources were higher in amplitude in the Nold than MCI group ($p < 0.000005$), and in the MCI than AD group ($p < 0.000005$ to 0.000001); these results disclosed the pattern Nold > MCI > AD for the parietal, occipital, temporal, and limbic alpha 1 and occipital alpha2 sources. Furthermore, frontal, temporal, and limbic delta sources were lower in amplitude in the Nold and MCI than in the AD groups ($p < 0.05$).

Figure 2 maps the grand average of the normalized LORETA solutions (i.e., relative power current density) modeling the distributed cortical EEG sources for delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma bands in the AD−, AD+, MCI−, MCI+ groups. Posterior alpha sources were generally higher in amplitude in the AD+ or MCI+ than AD− or MCI− group, whereas the opposite is true for the posterior delta sources.

Figure 3 plots the grand average of regional normalized LORETA solutions (i.e., relative power current density averaged with each ROI) relative to an ANOVA interaction ($F(90,5250) = 3.50$; $p < 0.00001$) among the factors Group (AD−, AD+, MCI−, MCI+), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic). Planned post-hoc testing indicated that occipital, temporal, and limbic alpha 1 sources were

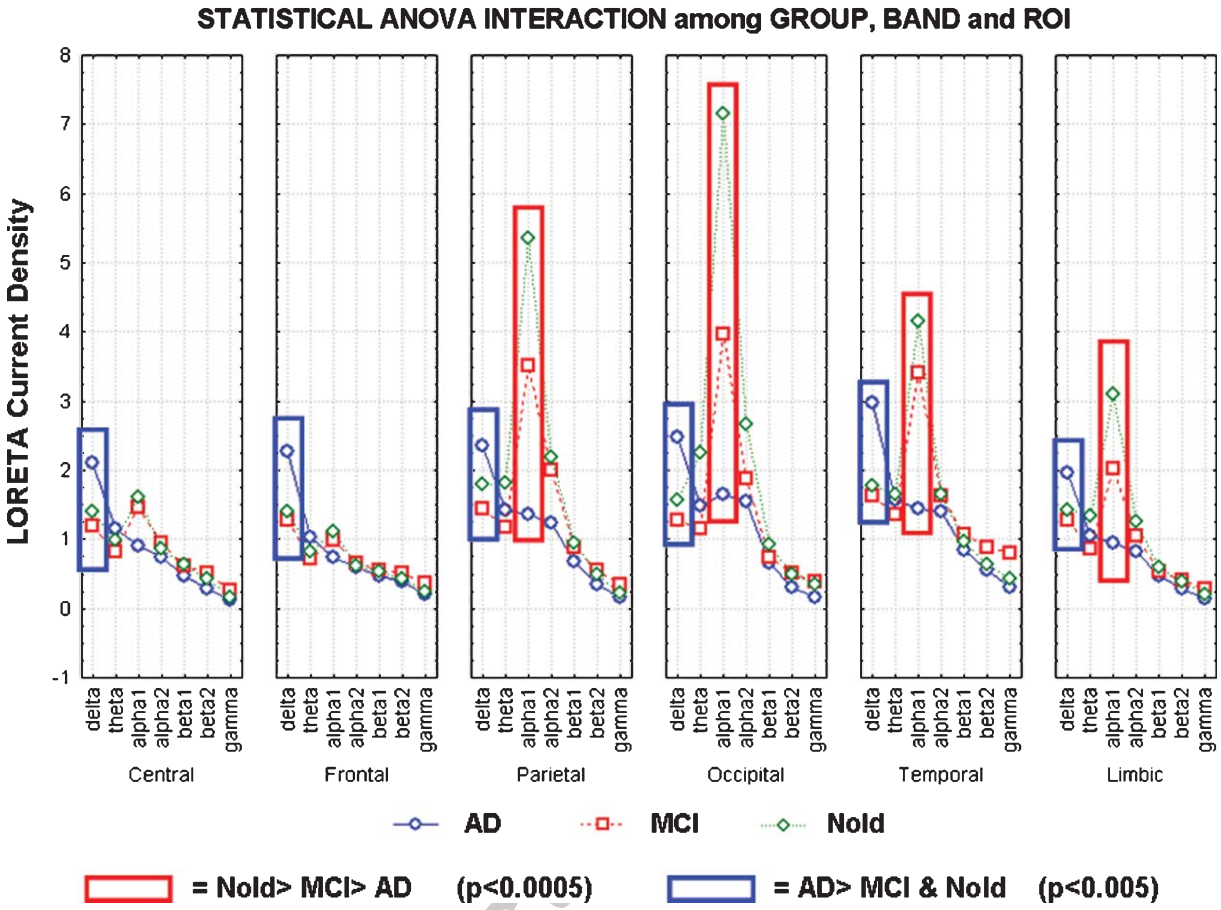

Fig. 1. Statistical ANOVA interaction ($F(60,6480) = 11.91$; $p < 0.0001$) among the factors Group (Nold, MCI, AD), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic).

higher in amplitude in the MCI+ than MCI− group ($p < 0.01$ to 0.000001). Furthermore, occipital delta sources were higher in amplitude in the AD− than AD+ group ($p < 0.01$). Finally, central, parietal, occipital, temporal, and limbic alpha sources were higher in amplitude in the AD+ than AD− group ($p < 0.05$ to 0.000001).

The mentioned delta and alpha sources showing statistically significant differences ($p < 0.05$) as a function of the white matter vascular lesions (i.e., MCI+ or AD+ versus MCI− or AD−) were correlated to MMSE score in the continuum of the MCI and AD subjects as a whole group. There was a positive correlation between MMSE score and any of the mentioned alpha sources at central (alpha 1, $r = 0.18$, $p = 0.02$; alpha 2, $r = 0.20$, $p = 0.008$), parietal (alpha 1, $r = 0.28$, $p = 0.0001$; alpha 2, $r = 0.32$, $p = 0.0001$), occipital (alpha 1, $r = 0.25$, $p = 0.001$; alpha 2, $r = 0.27$, $p = 0.0001$), temporal (alpha 1, $r = 0.31$, $p = 0.0001$; alpha 2, $r = 0.26$, $p = 0.001$), and limbic (alpha 1, $r = 0.30$, $p = 0.0001$; alpha 2, $r = 0.30$, $p = 0.0001$) macroregions. The higher the MMSE score, the higher the amplitude of alpha sources. Furthermore, there was a negative correlation between MMSE score and occipital delta sources ($r = -0.31$, $p = 0.0001$). The lower the MMSE score, the higher the amplitude of occipital delta sources.

Control analyses

We performed some control analyses to ascertain if the results of the main statistical analysis were affected by relevant confounding variables.

In a first control analysis, we tested whether the statistical results were influenced by the presence of ADNI and non-ADNI subjects in the MCI and AD groups. We divided the MCI and AD group in ADNI

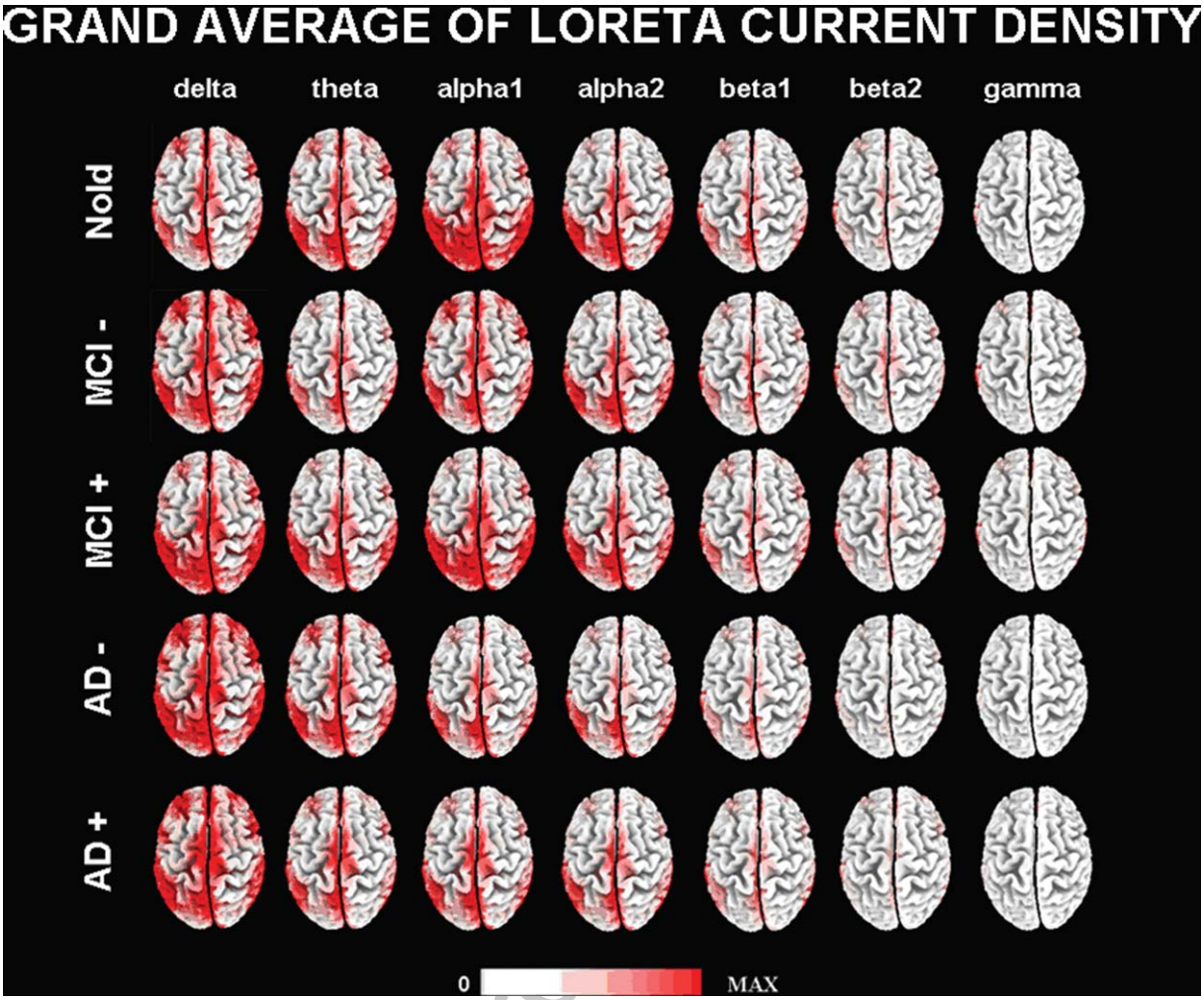

Fig. 2. Grand average of LORETA solutions (i.e., normalized relative current density at the cortical voxels) modeling the distributed EEG sources for delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma bands in Nold, MCI – (normalized white-matter vascular lesions < 4960), MCI+(normalized white-matter vascular lesions ≥4960), AD – (normalized white-matter vascular lesions < 3430), and AD+(normalized white-matter vascular lesions ≥3430) groups. The left side of the maps (top view) corresponds to the left hemisphere. Legend: LORETA, low resolution brain electromagnetic tomography. Color scale: all power density estimates were scaled based on the averaged maximum value (i.e., alpha 1 power value of occipital region in Nold).

(18 MCI and 50 AD) and non-ADNI (78 MCI and 33 AD). Statistical analysis of the LORETA source solutions showed no statistically significant difference ($F(1.91) = 0.01$; $p < 0.9235$) between MCI ADNI and MCI non-ADNI sub-groups. The same was true in AD patients, namely no statistically significant difference ($F(1.78) = 0.49$; $p < 0.4839$) between the AD ADNI and AD non-ADNI sub-groups.

In a second control analysis, we compared the Nold, MCI, and AD groups matched as number (40 AD, 40 MCI, and 40 Nold subjects), mean age (AD = 69.5 years; MCI = 69.8 years; Nold = 72.1 years), and mean IAF (AD = 8.6, MCI = 9.3, and Nold = 9.3 hertz). This allowed a good control of the inter-groups variability. The ANOVA design and covariates were those of the main ANOVA design. The ANOVA factors were Group (Nold, MCI, AD), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic). The results showed a statistically significant interaction among all factors ($F(60,3510) = 14.17$; $p < 0.0001$). As expected, ANOVA showed the well known abnormalities of delta and alpha sources, in detail the results disclosed the pattern Nold > MCI > AD for the parietal, occipital, and

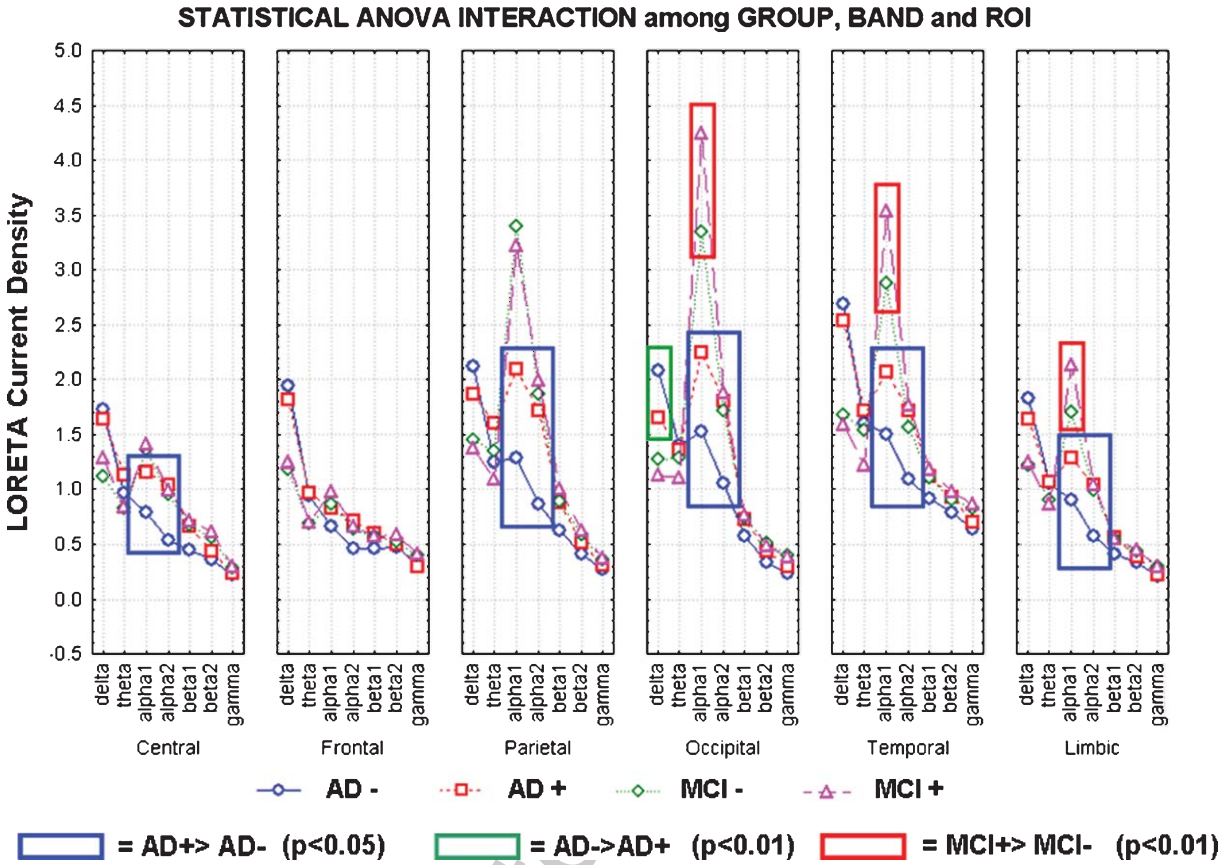

Fig. 3. Statistical ANOVA interaction ($F(90,5250) = 3.50$; $p < 0.00001$) among the factors Group (AD−, AD+, MCI−, MCI+), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (frontal, central, parietal, occipital, temporal, limbic).

limbic alpha and temporal alpha1 sources. Furthermore, frontal, occipital, and temporal delta sources were lower in amplitude in the Nold and MCI than in the AD groups ($p < 0.05$). These results globally confirmed those of the main analysis.

In a third control analysis, we compared white matter vascular lesions between MCI+ versus MCI− groups as well as between AD+ versus AD− groups. As expected, ANOVA showed that there were significantly higher values of the white matter vascular lesions in the MCI+ than MCI− ($p < 0.00001$) as well as in the AD+ than AD− subjects ($p < 0.00001$).

A fourth control analysis accounted for the variability of the MMSE score across the groups, although the MMSE score was used as a covariate in the main ANOVA design. We selected sub-groups of AD−, AD+, MCI−, and MCI+ subjects to minimize the difference of MMSE score between the AD− and AD+ groups as well as between the MCI− and MCI+groups (Table 4). The ANOVA design and covariates were those of the main ANOVA design. The ANOVA factors were Group (AD−, AD+, MCI−, MCI+; independent variable), Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma), and ROI (central, frontal, parietal, occipital, temporal, limbic). The results showed a statistically significant interaction among all factors ($F(90,2700) = 2.81$; $p < 0.00001$). Planned post-hoc testing indicated that central and temporal alpha 1 sources were higher in amplitude in the MCI+ than MCI− group ($p < 0.05$). Furthermore, occipital delta sources were lower in amplitude in the AD+ than AD− group ($p < 0.05$). Finally, central, parietal, occipital, and temporal alpha 1 sources were higher in amplitude in the AD+than AD− group ($p < 0.01$). These results globally confirmed those of the main ANOVA design.

DISCUSSION

In the present study, we tested the novel hypothesis that in AD subjects, resting state closed-eye EEG

Table 4
Demographic and clinical data of the subjects' sub-groups created to minimize the difference of MMSE between AD− and AD+ as well as between MCI− and MCI+

	Subjects (n)	Gender (M/F)	Age (years)	MMSE	IAF (Hz)	White matter vascular lesion (voxels)
MCI+	28	12/21	68.3 ± (1.5 SE)	26.3 ± (0.3 SE)	9.6 ± (0.2 SE)	7800 ± (713 SE)
MCI−	28	13/20	69.0 ± (1.1 SE)	26.2 ± (0.3 SE)	9.5 ± (0.2 SE)	2973 ± (227 SE)
AD+	19	8/11	72.1 ± (1.8 SE)	19.5 ± (0.8 SE)	8.8 ± (0.3 SE)	9795 ± (2403 SE)
AD−	19	7/12	70.1 ± (2.7 SE)	19.6 ± (0.8 SE)	8.4 ± (0.4 SE)	2231 ± (200 SE)

rhythms are not deteriorated due to the amount of white-matter vascular lesion, thus extending previous evidence in amnesic MCI subjects [1, 23]. To address this hypothesis, we estimated white matter vascular lesion with friendly and automated software (i.e., EMS tool of SPM) towards possible clinical applications. Such procedure was based on the use of PD, T1-, and T2-weighted MRIs recorded in amnesic MCI and AD subjects. Of note, we collected about 42% of the MRIs following the standards of the ADNI project (http://www.adni-info.org/), which aims at standardizing neuroimaging exams of aged people in multicenter AD studies on new markers and drugs. In this vein, we estimated cortical sources of resting state EEG rhythms by LORETA software, which can be freely downloaded by Internet (http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm), and has been successfully used by our Consortium in several field investigations [7, 14, 53–56].

In the present study, results of the control statistical analysis confirmed that the amnesic MCI subjects showed a decrease in amplitude of low-frequency alpha sources (8–10.5 Hz) compared to the Nold subjects. The AD subjects were characterized by an amplitude increase of delta sources (2–4 Hz), along with a strong amplitude reduction of low-frequency alpha sources. These findings are globally in line with previous evidence showing a pathological enhancement of the delta rhythms in AD subjects [7, 8, 76, 77], and a magnitude decrease of default alpha rhythms in MCI and/or AD subjects [2, 3, 7, 8, 15, 21, 22, 78]. Therefore, these control findings validated the present procedures for subjects' selection and EEG data analysis, thus corroborating the novel results on the relationships between resting state EEG sources and white matter vascular lesions in AD subjects.

Results of the main statistical analysis indicated that amplitude of posterior low-frequency alpha sources was higher in the amnesic MCI+ than MCI− group. As a novel finding, amplitude of occipital delta sources was lower in the AD+ than AD− group, whereas the opposite was true for central and posterior low- and high-frequency alpha sources. These results suggest that in AD subjects, central and posterior resting state delta and alpha rhythms are not deteriorated with the increase of white-matter vascular lesion, thus extending previous evidence on alpha sources in amnesic MCI subjects [1, 23].

Why were resting state EEG rhythms not deteriorated by the increase of white matter vascular lesions in amnesic MCI and AD subjects? To answer to this question, a brief overview on "normal" delta and alpha rhythms is helpful. In the condition of slow-wave sleep, corticofugal slow oscillations (<1 Hz) are effective in grouping thalamic-generated delta rhythms (1–4 Hz) and spindling activity (7–14 Hz) rhythms [79]. In the condition of brain arousal, spindles as well as high and low-frequency components of the delta rhythms are blocked by the inhibition of oscillators within, respectively, reticulo-thalamic (7–14 Hz), thalamo-cortical (1–4 Hz), and intracortical (<1 Hz) neuronal circuits. These rhythms are replaced by fast (beta and gamma) cortical oscillations, which are mainly induced by forebrain (nucleus basalis) cholinergic inputs to hippocampus and cortex as well as by thalamocortical projections [79, 80]. In the condition of awake rest, low-frequency (8–10.5 Hz) alpha would be mainly related to subject's global attentional readiness [72, 81–84] and would mainly reflect time-varying inputs of cortico-cortical and thalamo-cortical pathways [85]. Noteworthy, there is consensus that alpha rhythms represent the dominant resting oscillations of the adult, awake human brain [72, 81–84], and have been linked to intelligent quotient, memory, and cognition [52]. Keeping in mind this physiological premise, loss of synapses and neurons along the well known tracks of AD neurodegeneration [86, 87] may deteriorate the synchronization of cortical pyramidal neurons generating default alpha rhythms, and may disinhibit pathological delta rhythms in the condition of resting state. In this framework, diffuse white matter vascular lesions may not specifically

impair the neural circuits responsible for the transfer of signals into brain pathways that generate resting state alpha rhythms and inhibit pathological delta rhythms.

The present results support the notion that cerebrovascular and AD lesions do not represent additive or synergistic factors in the determination of the resting state EEG abnormalities during the evolution of the disease, although these lesions contribute to the development of cognitive impairment in AD patients [24, 26, 88]. In AD, cognitive and clinical conditions are affected by the severity of both neurodegenerative and cerebrovascular lesions in hippocampal, anterior cingulate gyrus, and parieto-temporal regions [89, 90–94]. Furthermore, these conditions depend on amyloid angiopathy of small vessels and on their structure/function [27–29]. Current evidence suggests that there is decreased vascular density in aging and AD, with a cerebrovascular dysfunction that precedes and accompanies cognitive dysfunction and neurodegeneration [95]. A decline in cerebrovascular angiogenesis typically inhibits recovery from hypoxia-induced capillary loss and cerebral blood flow may be inhibited by tortuous arterioles and deposition of excessive collagen in veins and venules [96]. In this framework, hypoperfusion may occurs early in AD, inducing white matter lesions and correlating with dementia [95–99]. However, resting state EEG abnormalities would be mainly affected by the AD neurodegenerative impairment of brain circuits, which may be not specifically targeted by diffuse white matter vascular lesions. Therefore, it can be speculated that resting state EEG rhythms might be more sensitive to neurodegenerative processes than cerebrovascular lesions in AD.

In conclusion, we tested whether cortical synchronization mechanisms at the basis of resting state EEG rhythms are abnormal in AD subjects, as a function of vascular lesion of white matter. The present results showed that in both amnesic MCI and AD subjects, posterior delta and alpha sources did not deteriorate with the increase of white matter vascular lesion, although white matter is known to be impaired along AD neurodegenerative process. These results need to be validated with a follow-up study evaluating resting state EEG rhythms and white matter vascular lesion. In principle, the present results suggest that abnormalities of resting state EEG rhythms might be related to AD neurodegeneration specifically impinging on the brain circuits generating these rhythms and cognitive status rather than to white matter vascular lesion globally affecting the whole brain.

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