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RESTING-STATE ELECTROENCEPHALOGRAPHIC DELTA RHYTHMS MAY REFLECT GLOBAL CORTICAL AROUSAL IN HEALTHY OLD SENIORS AND PATIENTS WITH ALZHEIMER'S DISEASE DEMENTIA

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Conflict of Interest Statement

None of the authors have potential conflicts of interest to be disclosed.

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Highlights (85-character limit per bullet)

- Resting EEG rhythms differ in Alzheimer's and healthy seniors (group level)
- Alzheimers' seniors show abnormally high delta and low alpha rhythms
- Delta and alpha power densities are negatively correlated in healthy seniors
- Above correlation is mitigated by Alzheimer's disease (dementia)

Journal Pre-proof

Abstract

Extending Başar's theory of event-related EEG oscillations, here we hypothesize that even in quiet wakefulness, transient increases in delta rhythms may enhance global cortical arousal as revealed by the desynchronization of alpha rhythms in normal (Nold) seniors with some derangement in Alzheimer's disease dementia (ADD).

Clinical and EEG datasets in 100 ADD and 100 Nold individuals matched as demography, education, and gender were taken from an international archive. Standard delta (< 4 Hz) and alpha1 (8-10.5 Hz) bands were used for the main analysis, while alpha2 (10.5-13 Hz), theta (4-8 Hz), beta1 (13-20 Hz), beta2 (20-35 Hz), and gamma (35-40 Hz) served as controls. In the interpretation, the higher the alpha1 power (density), the lower that arousal. As expected, when compared to the Nold group, the ADD group showed higher global (scalp) power density at the delta-theta band and lower global power density at the alpha-beta bands. As novel findings, we observed that: (1) in the Nold group, the global delta and alpha1-2 power were negatively and linearly correlated; (2) in the ADD group, this correlation was just marginal; and (3) in both Nold and AD groups, the EEG epochs with the highest delta power (median value for stratification) were associated with the lowest global alpha1 power. This effect was related to eLORETA freeware solutions showing maximum alpha1 source activations in posterior cortical regions.

These results suggest that even in quiet wakefulness, delta and alpha rhythms are related to each other, and ADD partially affects this cross-band neurophysiological mechanism.

Introduction

Earlier studies have applied the spectral analysis of resting state eyes-closed electroencephalographic (rsEEG) rhythms recorded at scalp electrodes in patients with Alzheimer's disease dementia (ADD), to understand the neurophysiological disease model towards potential clinical applications (Babiloni et al., 2020a). As compared to matched elderly seniors with normal cognition (Nold), ADD patients showed abnormalities in power density of rsEEG rhythms in vast scalp regions, namely there was higher power density at delta (<4 Hz) and theta (4-7 Hz) frequency bands, as well as low power density at alpha (8-12 Hz) and/or beta (13-20 Hz) frequency bands, these effects being especially consistent at delta and alpha frequencies (Babiloni et al., 2005a, Babiloni et al., 2017; Dierks et al., 1993, 2000; Huang et al. 2000; Jelic et al. 2000; Ponomareva et al., 2003; Jeong, 2004).

Other studies have successfully proved that those abnormalities at delta or alpha frequencies may be due to the effect of AD on neurophysiological oscillatory mechanisms. It was reported that rsEEG rhythms at delta and alpha frequencies were related to the atrophy of cortical gray matter in ADD patients (Babiloni et al., 2013,2015) and were partially normalized by a long-term administration of Acetylcholinesterase inhibitors (Kogan et al., 2001; Rodriguez et al., 2002; Babiloni et al., 2006a). Furthermore, they showed a strict relationship with typical AD-related genetic risk factors (Babiloni et al., 2006b,c).

It can be speculated that rsEEG rhythms at delta and alpha frequencies may reflect partially overlapping neurophysiological oscillatory mechanisms of synchronization of neural activity in subcortical, corticothalamic, and corticocortical circuits involved in the regulation of vigilance in quiet wakefulness at eyes closed (Hughes & Crunelli, 2005; Lörincz et al., 2008, 2009; Pfurtscheller and Lopes da Silva, 1999; Steriade and Llinas, 1988, Wan et al., 2019). In this neurophysiological frame, alpha rhythms may be dominant in occipital and parietal areas overlying visual and visuospatial cortical areas, as a reflection of the inhibition of those areas by the lack of input visual stimuli (Pfurtscheller and Lopes da Silva. 1999; Babiloni et al., 2020a,b). Other components of alpha rhythms may reflect the inhibition of auditory, somatosensory, and motor areas about a silent environment and a condition of psychophysiological relaxation (Pfurtscheller and Lopes da Silva. 1999; Babiloni et al., 2020a,b).

Less clear is the neurophysiological mechanism generating rsEEG rhythms at delta frequencies in quiet wakefulness. Traditionally, delta rhythms are low in amplitude in healthy adults during quiet wakefulness. At the same time, they show rapid transient enhancement during sensory event triggering global cortical excitation (arousal) and cognitive processes (Başar et al., 2016). Indeed, Dr Erol Başar's group has repeatedly shown that cognitive event-related EEG oscillations at delta and theta frequencies were dominant in healthy people and superimpose each other with given phases and amplitudes in the formation of the so-called event-related potentials (Basar et al., 2001; Karakaş et al., 2000). Furthermore, cognitive event-related EEG oscillations at delta and theta frequencies were attenuated in amplitude in ADD patients as compared to Nold seniors (Başar et al., 2016; Yener et al., 2007, 2008, 2009, 2012), even at the prodromal disease stage of mild cognitive impairment (Yener et al., 2016).

Another piece of the puzzle is that rsEEG rhythms at delta frequencies in quiet wakefulness may be an outcome of pathophysiological processes due to a loss of functional connectivity among thalamocortical and corticothalamic loops targeting widespread cortical modules in the regulation of quiet vigilance (Steriade and Llinas, 1988; Klimesch, 1999; Babiloni et al., 2010, 2020a,b).

Keeping in mind the above findings and considerations, the aim of the present exploratory study on archive data was to test the following hypotheses. As main hypotheses, we tested whether transient increases in delta rhythms during the quiet wakefulness may be related to an increment in the general cortical arousal, as measured by low-frequency alpha (alpha1) rhythms. This effect was expected in Nold seniors and may be deranged in ADD patients. Furthermore, the hypotheses of control analyses tested the topography and frequency contents of this effect and the possible effects of the involvement of several clinical units in the study. To test these hypotheses, clinical and rsEEG datasets in ADD and Nold individuals were taken from an international archive, and the relationship between those delta and alpha rhythms was investigated by the analytic procedures described in the following sections.

Noteworthy, the present study was mainly focused on resting state low-frequency alpha (alpha1) rhythms as they are dominant in the quiet wakefulness (resting state) and may be related to the fluctuation of persons' vigilance (Klimesch, 1999; Pfurtscheller and Lopes da Silva, 1999). Differently, high-frequency alpha rhythms (alpha2) may be more

related to the processing of modal specific or semantic information (Klimesch, 1999; Pfurtscheller and Lopes da Silva, 1999).

Materials and Methods

Subjects

In this study, we used the data of an international (Italy and Turkey) archive, formed by clinical and rsEEG data collected in 100 Nold seniors and 100 ADD patients. Those data were collected by the following qualified Italian and Turkish clinical units in the years 2010-2019 (i.e., all those units participated in this study): the Sapienza University of Rome (Italy), Institute for Scientific-Based Care and Research (IRCCS) Fatebenefratelli of Brescia (Italy), IRCCS SDN of Naples (Italy), IRCCS Oasi Maria SS of Troina (Italy), University of Genova (Italy), Hospital San Raffaele of Cassino (Italy), IRCCS San Raffaele Pisana of Rome (Italy), and Medipol University of Istanbul (Turkey). The present datasets were used in the following previous rsEEG studies of our research group to test other hypotheses about the rsEEG biomarkers of AD (Babiloni et al., 2013, 2015, 2016a, 2016b, 2017; Lizio et al., 2017).

The Nold seniors and ADD patients were carefully matched as to age, gender, and education. Table 1 summarizes the most relevant demographic (i.e., age, gender, and education) and clinical (i.e., mini-mental state evaluation, MMSE, score) features of those Nold and ADD participants. Furthermore, Table 1 reports the results of the presence or absence of statistically significant differences ($p < 0.05$) between the two groups as age (t-test), gender (Fisher test), education (t-test), and MMSE score (Mann Whitney U test). As expected, a statistically significant difference was found for the MMSE score ($p < 0.000001$), indicating a higher score in the Nold than the ADD group. No difference was found for the age, gender, and education between the groups ($p > 0.05$).

Local institutional Ethics Committee approved the present study. All experiments were performed with the informed and overt consent of each participant or caregiver, in line with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the local Institutional Review Board.

Insert here Table 1

Diagnostic criteria

Probable ADD was diagnosed based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR; American Psychiatric Association), and the National Institute of Neurological Disorders and Stroke-Alzheimer Disease and Related Disorders (NINCDS-ADRDA) working group (McKhann et al., 1984). The ADD individuals underwent medical, neuropsychological, neurological, and psychiatric evaluations including Instrumental Activities of Daily Living scale (IADL; Lawton and Brody, 1969), MMSE (Folstein et al., 1975), Clinical Dementia Rating (CDR; Hughes et al., 1982), Geriatric Depression Scale (GDS; Yesavage et al., 1982), and Hachinski Ischemic Score (HIS; Rosen et al., 1980).

Exclusion criteria included any kind of proof of other types or causes of dementia such as frontotemporal dementia (The Lund and Manchester Group, 1994), vascular dementia diagnosed based on the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) working group (Román et al., 1993), Parkinson disease (Gelb et al., 1999), Dementia with Lewy Bodies (McKeith et al., 2005), metabolic syndrome, nutritional deficits, tumors, etc.

Benzodiazepines, antidepressant and/or antihypertensive drugs were taken (when administered) after the EEG recording performed in the morning. While this procedure did not guarantee a full washout of the drug, it made comparable the timing of the drug administration across the ADD patients. Longer periods of suspension would not have been valid for obvious ethical reasons.

Most ADD patients (> 95%) followed a long-term therapy with daily doses of acetylcholinesterase inhibitors including donepezil, rivastigmine, or galantamine in standard dosages.

Nold participants underwent a cognitive screening as well as physical and neurological examinations to exclude any dementia or major cognitive deficits and psychiatric disorders. All Nold participants had a GDS score lower than the threshold of 5 (no depression) or no depression after an interview with a physician or clinical psychologist.

Participants affected by chronic systemic illnesses (diabetes mellitus, as an example) were excluded, as well as participants receiving chronic psychoactive drugs.

Participants with a history of earlier or present neurological or psychiatric diseases and drug or alcohol abuse were also excluded.

Finally, according to the Alzheimer's Disease Neuroimaging Initiative (ADNI; <http://adni.loni.usc.edu>), another exclusion criterion was a MMSE score below 27 for the Nold seniors and above 24 for the ADD patients.

rsEEG recordings

As a premise, this study is based on experiments performed in the framework of different clinical studies over time, so some methodological procedures were equal or remarkably similar across the participating clinical units, while others showed some differences. Furthermore, EEG procedures used in the present study are based on some assumptions, and the results must be interpreted as related to those assumptions. Specifically, we followed the procedures of preliminary rsEEG data analysis used in earlier investigations of our research group to enrich the interpretation of the present results (Lizio et al., 2016; Babiloni et al., 2015, 2016a, 2016b).

Instructions to participants for the rsEEG recordings were remarkably similar in all clinical units. All subjects were kindly asked to stay relaxed with the eyes closed during the experiments. They were also kindly asked not to move or talk and keep the mind wondering without focused mentalization. During the experimental recordings, the experimenters did control the subject's behavioral condition and on-going rsEEG traces, kindly helping the participants to keep the adequate level of vigilance (i.e., avoiding drowsiness and sleep onset). These alarms were annotated in the protocol for the phase of preliminary rsEEG data analysis.

In all experiments, at least 5 minutes of electrophysiological data were recorded by professional digital EEG systems licensed for clinical applications (i.e., EB-Neuro Be-light, Micromed, Brain vision, etc.) from 19 exploring scalp electrodes placed according to the 10–20 montage system (i.e., Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2). The ground electrodes were placed in the posterior midline. In contrast, the reference electrodes were located in different positions across participating clinical units (i.e., linked earlobes, mastoids, vertex, etc.) in line with local standard protocols and trials.

During the rsEEG recordings, electrode impedances were kept below 5 KOhm. The

rsEEG recordings were performed using 128 Hz or higher sampling rate (128-1,024 Hz) with an antialiasing bandpass between 0.01 Hz and 60-100 Hz.

In parallel to the rsEEG recording, bipolar vertical and horizontal electrooculographic (EOG) signals and one-channel electrocardiographic (ECG) signals were also acquired using the same sampling frequency adopted to record the rsEEG data (128-1,024 Hz). Consequently, rsEEG, EOG, and ECG signals had the same sampling rate, so EOG and ECG signals could be used for the artifact detection and their off-line correction when adequate.

As mentioned above, some rsEEG datasets were recorded using a relatively low sampling frequency of 128 Hz (< 20% of the rsEEG datasets collected). It should be remarked that such a sampling frequency is sub-optimal for an ideal reconstruction of rsEEG signal beyond 40 Hz without aliasing. Ideally, a factor of 3-4 between the low-band pass limit and the rsEEG sampling frequency should be set.

Preliminary rsEEG data analysis

In the preliminary analysis, the rsEEG data were split into 2-s epochs and analyzed off-line centrally. This segmentation allowed the use of standard toolboxes for the spectral analysis of rsEEG activity such as Fast Fourier Transform (FFT) and eLORETA source estimation, which assume the stationarity of rsEEG activity. Furthermore, it allowed minimizing the rejection of rsEEG data around a short of a period of artifactual activity. The use of those procedures allowed a better understanding of the present results in light of previous reference evidence of our research line (Lizio et al., 2016; Babiloni et al., 2015, 2016a, 2016b), but it implied the focus on the linear components of rsEEG signals.

Two independent experimenters (G. N. and R. L.) performed a visual analysis of EOG and rsEEG data blind to the clinical diagnosis associated with the electrophysiological datasets. They rejected those with artifacts due to instruments, electronic noise, head-neck movements, and face muscle tension. They also rejected rsEEG epochs with amplitude values exceeding 100 μ V. Particular attention was given to the contamination of ocular activity (i.e., blinking) on electrophysiological data recorded at frontal (i.e., F7, F3, Fz, F4, and F8) and frontopolar (Fp1 and Fp2) electrodes. This specific exam was based on the comparison of EOG and rsEEG traces. The rsEEG epochs with artifacts marked as eye movements and blinking were given as inputs to a software toolbox

based on an autoregressive model for their possible correction. Technical details and performances of this procedure were reported elsewhere (Moretti et al., 2003). Noteworthy, the outcome of this procedure was visually revised by the two experimenters (G. N. and R. L.). All Nold and ADD datasets showed less than 15% of artifact-free rsEEG epochs, without significant differences between the Nold and ADD groups (t-test, $p > 0.05$).

To harmonize rsEEG data recorded using different reference electrodes and sampling frequency rates, artifact-free rsEEG epochs were off-line frequency-band passed at 0.1-45 Hz and down sampled, when appropriate, to make the sampling rate of all artifact-free rsEEG datasets in the Nold and ADD participants equal to 128 Hz. For the sake of harmonization of all datasets, the recorded rsEEG data were re-referenced to the common average reference.

Scalp power density of rsEEG rhythms

For each ADD and Nold participant, the global normalized rsEEG power density at the scalp electrode level was evaluated. In detail, the procedure was performed as follows:

- 1) A standard digital FFT-based power spectrum analysis (Welch technique, Hanning windowing function, no phase shift) computed the absolute scalp power density of rsEEG rhythms with 0.5 Hz of frequency resolution at each electrode (i.e., 19 electrodes of the 10-20 montage system) and frequency bin (i.e., 0.5-45 Hz) from all artifact-free rsEEG epochs.
- 2) The scalp rsEEG power density at each electrode and frequency bin was normalized to the mean value obtained averaging the scalp rsEEG power density across all frequency bins and scalp electrodes.
- 3) The “global” scalp normalized rsEEG power density at each frequency bin was calculated averaging the normalized scalp rsEEG power density values across all 19 electrodes of 10-20 montage system.
- 4) The global scalp normalized rsEEG power density values at each frequency band of interest were averaged to obtain the frequency band values. The following standard frequency bands of interest were considered, in line with previous relevant rsEEG studies (Chiaramonti et al., 1997; Besthorn et al., 1997; Lizio et al., 2016; Galluzzi et al., 2016; Del Percio et al., 2019; Babiloni et al., 2015, 2016a, 2016b, 2020): 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). These frequency bands were computed sharing the frequency bin by two contiguous bands, as a widely accepted procedure (Besthorn et al., 1997) that fits the theoretical consideration that near EEG rhythms may overlap at their frequency borders (Klimesch et al., 1996; Klimesch, 1999; Babiloni et al., 2015, 2016a, 2016b, 2016c, 2020).

Evaluation of rsEEG epochs showing highest (DELTA+) and smallest (DELTA-) delta power density

For each ADD and Nold participant, the artifact-free rsEEG epochs were classified into 2 subgroups based on the median value of the global Delta scalp power density. One subgroup included 50% of the rsEEG epochs with the highest values of the global scalp normalized rsEEG power density at the delta band (i.e., high delta subgroup, DELTA+). In contrast, the other included 50% of the rsEEG epochs with the smallest values of that power density (i.e., low delta subgroup, DELTA-).

Cortical sources of rsEEG epochs as computed by eLORETA

The official freeware tool 'exact' LORETA (eLORETA) implemented by Pascual-Marqui (2007a) was used for the linear estimation of the cortical source activity generating scalp-recorded rsEEG rhythms. The official implementation of eLORETA uses a spherical head volume conductor model composed of the scalp, skull, and brain. In the scalp compartment, exploring electrodes can be virtually positioned to give EEG data as an input to the source estimation (Pascual-Marqui, 2007). The brain model is based on a realistic cerebral shape taken from a template typically used in the neuroimaging studies, namely that of the Montreal Neurological Institute (MNI152 template). The eLORETA freeware solves the so-called EEG inverse problem estimating “neural” current density values at any cortical voxel of the mentioned spherical head volume conductor model. The solutions are computed rsEEG frequency bin-by-frequency bin. The input for this estimation is the EEG spectral power density computed at 19 scalp electrodes of the 10-20 montage system.

In the eLORETA package, electrical brain source space is formed by 6,239 voxels with 5 mm resolution, restricted to the cortical gray matter of the spherical head volume conductor model. An equivalent current dipole is located in each voxel. For each voxel, the

eLORETA package supplies the Talairach coordinates, the lobe, and the Brodmann area (BA).

For each frequency bin, eLORETA source estimates are expressed as current density values for any dipole vector of a given voxel (x, y, and z vectors). For each voxel, these three values were averaged as an estimate of neural current density at that voxel for a given frequency bin.

The eLORETA estimates of neural current density were normalized by the following procedure. We averaged eLORETA source estimates across all frequency bins from 0.5 to 45 Hz and 6,239 voxels of the brain model volume, to obtain the eLORETA “mean” neural current density. Afterwards, we computed the ratio between any original eLORETA estimate of the neural current density at a given frequency bin/voxel and the eLORETA “mean” neural current density. As a result, any original eLORETA estimate of the neural current density at a given frequency bin/voxel changed to a normalized eLORETA source estimate.

In line with the general low spatial resolution of the present EEG methodological approach (i.e., 19 scalp electrodes), we performed a regional analysis of the eLORETA solutions. For this purpose, we collapsed the eLORETA source estimates within bilateral frontal, central, temporal, parietal, occipital, and limbic macroregions (ROIs) considered separately. Table 2 reports the list of the BAs used for the ROIs considered in the present study.

Insert here Table 2

Statistical analysis

All statistical sessions were performed by the commercial tool STATISTICA 10 (StatSoft Inc., www.statsoft.com). In all statistical sessions, the use of parametric linear models (i.e., ANOVA or Pearson test) implies that dependent variables approximate Gaussian distributions while global scalp normalized rsEEG power density and regional normalized eLORETA current density distributions may not fit this feature. To tackle this issue, we computed the log 10 transformation of the global scalp normalized rsEEG power density and the regional normalized eLORETA current density distributions. Afterwards, we confirmed their Gaussian distribution by the Kolmogorov-Smirnov test ($p > 0.05$).

In all ANOVAs, Mauchly's test evaluated the sphericity assumption, and the degrees of freedom were corrected by the Greenhouse-Geisser procedure when appropriate ($p < 0.05$). Duncan test was used for post-hoc comparisons using a statistical threshold of $p < 0.05$ one-tail, which was Bonferroni corrected for the planned contrasts.

The results of the statistical analyses testing the main hypotheses of the present study were controlled by the iterative (leave-one-out) Grubbs' test. This test controls for the presence of one or more outliers in the distribution of the significant dependent variables. The null hypothesis of the non-outlier status was tested at the arbitrary threshold of $p > 0.001$ to remove only individual values with high probability to be outliers.

Main hypotheses

Three within-group statistical sessions were performed to evaluate the main hypotheses predicting a relationship between the global scalp (normalized) delta and alpha1 power density in the Nold seniors, and the possible derangement of this relationship in the ADD patients.

The first session used Pearson test to test whether the global scalp delta and alpha1 power density may show a negative and linear correlation across subjects in the Nold and ADD groups, considered separately ($p < 0.05$ corrected = $p < 0.05/2 = 0.025$).

The second session included 2 ANOVAs, namely one for the Nold group and the other for the ADD group. These ANOVAs tested the relationship between the global scalp delta and alpha power density without the assumption of linearity. Specifically, the ANOVAs tested whether as compared to the 50% of the (artifact-free) rsEEG epochs with the lowest global scalp delta power density (DELTA-), the 50% of the (artifact-free) rsEEG epochs with the highest global scalp delta power density (DELTA+) may be associated with the lowest global scalp alpha1 power density ($p < 0.05$). The global scalp rsEEG power density served as a dependent variable. The ANOVA factors were Delta (DELTA+ and DELTA-) and Band (theta, alpha1, alpha2, beta1, beta2, and gamma). The hypothesis would be confirmed by the following results: (1) a statistically significant ANOVA interaction between the factors ($p < 0.05$) and (2) a post-hoc Duncan test indicating statistically significant differences in the alpha1 power density between the DELTA+ and DELTA- levels (i.e., $DELTA+ \neq DELTA-$; Bonferroni corrected $p < 0.05$, the Bonferroni correction was applied for 6 frequency bands, $p < 0.05/6 = 0.0083$).

The third session was performed to test whether the linear correlation between the global scalp delta and alpha1 power density may be higher in the DELTA- than the DELTA+ rsEEG epochs, considered separately. The analysis was performed in both Nold and ADD groups, considered separately. To address this issue, Pearson test ($p < 0.05$ Bonferroni corrected; $p < 0.05/4 = 0.0125$) was used.

Furthermore, 2 between-group statistical sessions were performed to evaluate whether the negative association between the global scalp delta and alpha1 power density in the above analysis may differ between the Nold and the ADD groups.

The first session was performed to test whether the linear correlation between delta and alpha1 power density may be higher in the Nold than the ADD group. To this aim, Fisher r-to-z transformation ($p < 0.05$) was performed between the 2 r coefficients of correlation between the delta and alpha1 power density, and these output values were compared between the Nold and ADD groups.

The second session was performed to test whether the decrease of the global scalp alpha1 power density in the DELTA- over the DELTA+ rsEEG epochs may be higher in the Nold than the ADD group. To this aim, the following procedure was performed: (1) for each Nold and ADD participant, the difference in the global scalp alpha1 power density between the DELTA+ and DELTA- rsEEG epochs (i.e., DELTA+ minus DELTA-) was computed; (2) the DELTA+ minus DELTA- values were squared-root transformed to make them Gaussian; and (3) a t-test ($p < 0.05$) was performed to evaluate whether that difference in the global scalp normalized alpha1 power density (DELTA+ minus DELTA-) may be higher in the Nold than the ADD group.

Hypotheses of the control analyses

Three statistical sessions tested the hypotheses of the control analyses aimed to corroborate the results of the analyses testing the main hypotheses about the relationship between the global scalp (normalized) delta and alpha power density in the Nold seniors, and the possible derangement of this relationship in the ADD patients.

The first session tested the hypothesis that in the present global scalp delta and alpha1 power density may be abnormal in the ADD patients as compared to the Nold seniors ($p < 0.05$). In an ANOVA design, the global scalp rsEEG power density served as a dependent variable ($p < 0.05$). The ANOVA factors were Group (Nold and ADD) and Band (delta, theta, alpha1, alpha2, beta1, beta2, and gamma). The hypothesis would be

confirmed by the following results: (1) a statistically significant ANOVA interaction between the two factors ($p < 0.05$) and (2) a post-hoc Duncan test indicating statistically significant differences in the global scalp delta and alpha1 power density between Nold and ADD groups (i.e., $Nold \neq ADD$; Bonferroni corrected $p < 0.05$, the Bonferroni correction was applied for 7 frequency bands, $p < 0.05/7 = 0.007$).

The second session tested whether the results of the main statistical analysis may be affected by the inclusion of different clinical units (i.e., Sapienza University of Rome, IRCCS Fatebenefratelli of Brescia, IRCCS SDN of Naples, IRCCS Oasi Maria SS of Troina, University of Genova, Hospital San Raffaele of Cassino, IRCCS San Raffaele Pisana of Rome, and Medipol University of Istanbul) and EEG systems (EB-Neuro Be-light and Brain vision). To this aim, we repeated the main statistical sessions using the different clinical units and EEG systems as covariates. Briefly, we computed: (1) a partial correlation ($p < 0.05$ Bonferroni corrected $p < 0.05/2 = 0.025$) between the global scalp normalized delta and alpha1 power density in the Nold and ADD groups, considered separately; (2) 2 ANOVAs (one for the Nold group and the other for the ADD group; $p < 0.05$) having the global scalp normalized rsEEG power density as a dependent variable and Delta (DELTA+ and DELTA-) and Band (theta, alpha1, alpha2, beta1, beta2, and gamma) as factors. Bonferroni correction was applied for 6 frequency bands, $p < 0.05/6 = 0.0083$; (3) 2 ANOVAs (one for the Nold group and the other for the ADD group; $p < 0.05$) having the regional normalized alpha1 rsEEG current density as a dependent variable and Delta (DELTA+ and DELTA-) and ROI (frontal, central, parietal, occipital, temporal, and limbic) as factors. Bonferroni correction was applied for 6 ROIs, $p < 0.05/6 = 0.0083$.

The third session evaluated whether the correlation between the global scalp normalized delta and alpha1 power density may be biased by choice of standard fixed frequency bands to represent alpha rhythms. Indeed, that choice did not account for the significant within- and between-group variability in the so-called individual alpha frequency peak (IAFp), defined as the frequency associated with the strongest rsEEG power density at the extended alpha range of 7 - 13 Hz (Klimesch et al., 1999). To this aim, the following procedure was performed. Firstly, for each Nold and ADD participant, the IAF was identified. As a result, the mean IAFp was 9.2 Hz (± 0.1 SE) in the Nold group and 7.9 Hz (± 0.2 SE) in the ADD group. As expected, a t-test showed that the mean IAFp was greater in the Nold than the ADD group ($p < 0.000001$). Secondly, based on the IAFp, the individual alpha frequency band ranges were defined as follows: alpha1 from IAF-1 to

IAF Hz and alpha2 from IAF to IAF + 2 Hz. Consequently, in the Nold group, we defined the individual alpha1 band from 7 to 9 Hz and the individual alpha2 band from 9 to 11 Hz. In the ADD group, the individual alpha1 band was set from 6 to 8 Hz and the individual alpha2 band from 8 to 10 Hz. Thirdly, the global scalp (normalized) alpha1 and alpha2 power density were computed following the procedure described in the “*Scalp power density of rsEEG rhythms*” section of “Materials and Methods.” These resulting power density values were log 10 transformed to make them Gaussian. Fourthly, Pearson test ($p < 0.05$ Bonferroni corrected; $p < 0.05/4 = 0.0125$) was used to evaluate the correlation between the global scalp (normalized) delta power density (i.e., 2-4 Hz) and both the individual global scalp alpha1 and alpha2 power density in the Nold and ADD groups, considered separately.

Finally, another statistical session tested the hypothesis that the main effects of the global scalp delta power density may occur in posterior cortical (eLORETA) sources of alpha1 rhythms. Two ANOVAs were used, namely one for the Nold group and the other for the ADD group. The eLORETA current density served as a dependent variable. Specifically, the ANOVA designs tested whether as compared to the DELTA- rsEEG epochs, the 50% of the DELTA+ rsEEG epochs may be associated with the lowest eLORETA alpha1 source activations in posterior cortical regions ($p < 0.05$). The ANOVA factors were Delta (DELTA+ and DELTA-) and ROI (frontal, central, parietal, occipital, temporal, and limbic). The hypothesis would be confirmed by the following results: (1) a statistically significant ANOVA interaction between the two factors ($p < 0.05$) and (2) a post-hoc Duncan test indicating statistically significant differences between the DELTA+ and DELTA- rsEEG epochs in the alpha1 normalized eLORETA current density estimated in posterior ROIs (i.e. $\text{DELTA+} \neq \text{DELTA-}$; Bonferroni corrected $p < 0.05$, the Bonferroni correction was applied for 6 ROIs, $p < 0.05/6 = 0.008$). Furthermore, a voxel-based topographical control analysis of these eLORETA solutions was performed ($p < 0.05$ corrected). The general methodology and results of this analysis are reported in the Supplementary Materials.

Results

Results of the statistical analyses testing the main study hypotheses

Figure 1 displays the scatterplots showing the global scalp normalized rsEEG power density values (log 10 transformed) at the delta and alpha1 bands computed in the Nold

and ADD groups, considered separately. Pearson test ($p < 0.05$ corrected = 0.025) unveiled a statistically significant negative correlation between the delta and alpha1 power density in the Nold group ($r = -0.60$; $p = 0.000001$). The higher the global scalp delta power density, the lower the global scalp alpha1 power density as a reflection of the cortical arousal. Noteworthy, that correlation was just marginal in the ADD group ($r = -0.18$; $p = 0.07$).

Insert here Figure 1

Figure 2 (top) shows the grand average of the global scalp normalized rsEEG power density values (log 10 transformed) in the Nold group relative. The data refer to a statistically significant ANOVA interaction effect ($F = 44.3$; $p < 0.000001$) between the factors Delta (DELTA+ and DELTA-) and Band (theta, alpha1, alpha2, beta1, beta2, and gamma). The Duncan planned post-hoc testing ($p < 0.05$ corrected = $p < 0.008$) showed that the discriminant pattern DELTA+ < DELTA- was fitted by the global scalp normalized rsEEG power density values at the alpha1 ($p < 0.000001$), alpha2 ($p < 0.000001$), beta1 ($p < 0.000001$), beta2 ($p < 0.000001$), and gamma ($p < 0.001$) bands. These values were lower for the DELTA+ than the DELTA- rsEEG epochs.

Similarly, Figure 2 (bottom) shows the grand average of the global scalp normalized rsEEG power density values (log 10 transformed) in the ADD group. The data refer to a statistically significant ANOVA interaction effect ($F = 50.1$; $p < 0.000001$) between the factors Delta (DELTA- and DELTA+) and Band (theta, alpha1, alpha2, beta1, beta2, and gamma). The Duncan planned post-hoc testing ($p < 0.008$) showed that the discriminant pattern DELTA+ < DELTA- was fitted by the global scalp normalized rsEEG power density values at the theta ($p < 0.000001$), alpha1 ($p < 0.000005$), alpha2 ($p < 0.000001$), beta1 ($p < 0.000001$), beta2 ($p < 0.000001$), and gamma ($p < 0.001$) bands. As for the Nold group, these values were lower for the DELTA+ than the DELTA- rsEEG epochs.

Insert here Figure 2

Figure 3 shows the results of the analysis testing whether the linear correlation between the global scalp normalized delta and alpha1 power density may be higher in the rsEEG epochs with the lowest (DELTA-) than the highest (DELTA+) global scalp delta normalized power density, considered separately. The analysis was performed in the Nold and ADD groups. In the Nold group, Pearson test ($p < 0.05$ corrected = 0.0125) unveiled a

statistically significant negative correlation between the delta and alpha1 power density for both DELTA- ($r = -0.66$; $p = 0.000001$) and DELTA+ ($r = -0.56$; $p = 0.000001$) rsEEG epochs. In the ADD group, such a statistically significant negative correlation was found only for the DELTA- rsEEG epochs ($r = -0.33$; $p = 0.001$). In both Nold and ADD groups, the r coefficient of correlation between the global scalp delta and alpha1 power density was higher in the DELTA- than the DELTA+.

Insert here Figure 3

Significant effects ($p < 0.05$) were also found in the 2 between-group statistical sessions evaluating whether the negative association between the global scalp (normalized) delta and alpha1 power density in the main analysis may differ between the Nold and ADD groups. In the first session, Fisher r -to- z transformation ($p < 0.05$) showed that the r coefficient of correlation between the global scalp delta and alpha1 power density was significantly higher in the Nold group as compared to the ADD group ($Z = 3.56$; $p = 0.0003$). In the second session, t -test ($p < 0.05$ squared-root transformed data) showed that the difference of the global scalp normalized alpha1 power density between the DELTA+ and DELTA- epochs (DELTA+ minus DELTA-) did not differ in the Nold group as compared to the ADD group ($p > 0.05$).

The above results of the main statistical analyses were not due to outliers in the rsEEG variables used, as shown by Grubbs' test with an arbitrary threshold of $p > 0.001$. On the whole, the results suggest that the relationship between the global scalp normalized delta and alpha1 power density computed in the Nold group is partially affected in the ADD group.

Results of the control analyses

Concerning the control of the ADD-related abnormalities in rsEEG delta and alpha1 rhythms, Figure 4 shows the grand average of the global scalp normalized rsEEG power density values (log 10 transformed). These data refer to a statistically significant ANOVA interaction effect ($F = 36.6$; $p < 0.00001$) between the factors Group (Nold and ADD) and Band (delta, theta, alpha1, alpha2, beta1, beta2, and gamma). In the Nold group as a physiological reference, the dominant value of the global scalp normalized rsEEG power density was seen at the alpha1 band. Low values of the global scalp normalized rsEEG power density were found at the delta, theta, and alpha2 bands. The global scalp

normalized rsEEG power density values at the beta1, beta2, and gamma bands were extremely low. As compared to the Nold group, the ADD group showed a substantial decrease in the delta and theta global scalp normalized rsEEG power density values. Furthermore, the ADD group showed a substantial increase in the alpha and beta global scalp normalized rsEEG power density values.

The Duncan planned post-hoc testing ($p < 0.007$) showed that the discriminant pattern Nold < ADD was fitted by the delta ($p < 0.0001$) and theta ($p < 0.0005$) global scalp normalized rsEEG power density values. Furthermore, the discriminant pattern Nold > ADD was fitted by the alpha1 ($p < 0.000005$), alpha2 ($p < 0.00001$), and beta1 ($p < 0.005$) global scalp normalized rsEEG power density values.

Insert here Figure 4

Concerning the possible confounding effect of multiple clinical units (i.e., Sapienza University of Rome, IRCCS Fatebenefratelli of Brescia, IRCCS SDN of Naples, IRCCS Oasi Maria SS of Troina, University of Genova, Hospital San Raffaele of Cassino, IRCCS San Raffaele Pisana of Rome, and Medipol University of Istanbul) and EEG systems (EB-Neuro Be-light and Brain vision), all control analyses showed results in line with those of the main analyses. Specifically, (1) the partial correlation analysis confirmed the statistically significant negative correlation between the global scalp normalized delta and alpha1 power density computed in the Nold ($r = -0.64$; $p = 0.000001$) and ADD ($r = -0.23$; $p = 0.02$) groups, considered separately; (2) the 2 ANOVAs (one for the Nold group and the other for the ADD group; $p < 0.05$) using the global scalp normalized rsEEG power density as a dependent variable confirmed the statistically significant ANOVA interaction effect (Nold $F = 16.5$, $p < 0.0001$; ADD: $F = 12.3$, $p < 0.0001$) between the factors Delta (DELTA+ and DELTA-) and Band (theta, alpha1, alpha2, beta1, beta2, and gamma). The Duncan planned post-hoc testing ($p < 0.0083$) showed that the discriminant pattern DELTA+ < DELTA- was fitted by the global scalp normalized rsEEG power density values at the alpha1, alpha2, beta1, beta2, and gamma bands in the Nold group ($p < 0.005$ to $p < 0.00001$) and theta, alpha1, alpha2, beta1, beta2, and gamma bands in the ADD group ($p < 0.001$ to $p < 0.00001$); and (3) the 2 ANOVAs (one for the Nold group and the other for the ADD group; $p < 0.05$) using the eLORETA regional normalized alpha1 current density as a dependent variable confirmed the statistically significant ANOVA interaction effect (Nold $F = 11.1$, $p < 0.0001$; ADD: $F = 5.1$, $p < 0.0005$) between the

factors Delta (DELTA+ and DELTA-) and ROI (frontal, central, parietal, occipital, temporal, and limbic). The Duncan planned post-hoc testing ($p < 0.0083$) showed that the discriminant pattern DELTA+ < DELTA- was fitted by the eLORETA regional normalized alpha1 current density in the central, parietal, occipital, temporal, and limbic regions in the Nold group ($p < 0.0001$ to $p < 0.00001$), while that effect was observed in the frontal, central, parietal, occipital, temporal, and limbic regions in the ADD group ($p < 0.0001$ to $p < 0.000005$).

Concerning the potential confounding effects of the fixed alpha frequency bands, Figure 5 shows the scatterplots showing the relationship between the global scalp normalized delta power density (log 10 transformed) and the global scalp normalized alpha1 and alpha2 power density computed on an individual basis in the Nold and ADD groups, considered separately (see Materials and Methods for the procedure to define individual alpha1 and alpha2). In the Nold group, Pearson test ($p < 0.0125$) unveiled a statistically significant negative correlation between the global scalp normalized delta power density and the global scalp normalized power density at both individual alpha1 ($r = -0.36$; $p = 0.0002$) and alpha2 ($r = -0.60$; $p = 0.000001$) bands. The lower the global scalp normalized delta power density, the higher the global scalp normalized rsEEG power density at those individual alpha bands. In the AD group, that correlation was just marginal, namely delta vs. individual alpha2 returned $r = -0.18$ ($p = 0.07$). Overall, the results globally confirmed the main results obtained with the fixed alpha bands.

Insert here Figure 5

Concerning the topographical effects of the global delta on the alpha1 rhythms, we performed an exploratory control analysis using eLORETA source estimation. Figure 6 displays the grand average of the eLORETA regional normalized alpha1 current density (log 10 transformed) estimated in the Nold and ADD groups, considered separately. In the Nold group (Figure 6, top), these data refer to a statistically significant ANOVA interaction effect ($F = 24.1$; $p < 0.00001$) between the factors Delta (DELTA+ and DELTA-) and ROI (frontal, central, parietal, occipital, temporal, and limbic). The Duncan planned post-hoc testing ($p < 0.008$) showed that the discriminant pattern DELTA+ < DELTA- was fitted by the eLORETA regional normalized alpha1 current density values in the central ($p < 0.00001$), parietal ($p < 0.00001$), occipital ($p < 0.00001$), temporal ($p < 0.00001$), and

limbic ($p < 0.00001$) ROIs. These values were lower for the DELTA+ than the DELTA-rsEEG epochs.

Similar effects were observed in the ADD group (Figure 4, bottom) with a statistically significant ANOVA interaction effect ($F = 10.9$; $p < 0.00001$) between the factors Delta (DELTA+ and DELTA-) and ROI (frontal, central, parietal, occipital, temporal, and limbic). The Duncan planned post-hoc testing ($p < 0.008$) showed that the discriminant pattern DELTA+ < DELTA- was fitted by the eLORETA regional normalized alpha1 current density values in the frontal ($p < 0.0001$), central ($p < 0.00001$), parietal ($p < 0.000005$), occipital ($p < 0.00001$), temporal ($p < 0.000005$), and limbic ($p < 0.00001$) ROIs. As for the Nold group, these values were lower for the DELTA+ than the DELTA-rsEEG epochs.

Insert here Figure 6

Supplementary materials report the voxel-based mapping of the eLORETA regional normalized alpha1 current density values showing a finer localization of the maximum differences between DELTA+ and the DELTA-rsEEG epochs in the Nold and ADD groups. This localization was observed in the occipital lobe in the Nold group and the central areas in the ADD group. These results are illustrated in Supplementary Figures A and B.

In the whole, the above results of the control analyses corroborated those of the main analyses.

Discussion

In this exploratory rsEEG study on archive data, the main hypotheses tested whether (1) Nold seniors may show a relationship between delta and low-frequency alpha (alpha1) rhythms during the quiet wakefulness and (2) this relationship may be deranged in ADD patients. The main results can be summarized as follows. In the Nold group, the global delta (< 4 Hz) and alpha1 (8-10.5 Hz) power density were negatively and linearly correlated across the individuals. The higher the delta power density, the lower the alpha1 power density as a sign of tonic cortical arousal. In the ADD group, such a correlation was just marginal. In contrast, both Nold and ADD groups similarly showed that the global alpha1 power density was lower within each group when computed from the rsEEG epochs with the highest as compared to the lowest delta power density.

Overall, these results suggest that in the Nold seniors, the fluctuation of the resting state delta rhythms may be inversely associated with the alpha rhythms, possibly modulating the cortical arousal in the quiet wakefulness. In the ADD patients, this neurophysiological mechanism of cross-frequency amplitude coupling may be partially affected by individuals when all rsEEG epochs were considered. At the same time, it was preserved the different effect of the rsEEG epochs with the highest (DELTA+) vs. the lowest (DELTA-) global delta power density on the alpha1 power density.

To understand better the complex effect observed in the ADD patients, we performed a control analysis. Specifically, we computed the correlation across individuals between the delta and alpha1 rhythms for the rsEEG epochs showing DELTA+ and DELTA-, considered separately. The results were insightful. In the Nold group, the global delta and alpha1 power density were correlated considering either the DELTA+ or the DELTA-rsEEG epochs. In contrast, the ADD group showed the correlation only when the DELTA-rsEEG epochs were used. This control result suggests that in ADD patients, the typical increase in resting state delta rhythms due to the disease may partially derange the physiological correlation between the resting state delta and alpha rhythms observed in the present Nold seniors.

In this explanatory line, other control analyses showed that the current findings were affected by neither the frequency slowing of the alpha rhythms in the ADD over the Nold individuals nor the inclusion of several clinical units in the experiments. Furthermore, as expected, the ADD group pointed to an abnormal diffuse increase in the resting state delta (and theta) rhythms and an abnormal decrease in the alpha (and beta) rhythms. Finally, an explorative control analysis of rsEEG (eLORETA) source activation showed that both the Nold and ADD groups displayed lower posterior alpha1 source activations in the DELTA+ than the DELTA- rsEEG epochs. Noteworthy, the ADD group was characterized by a topographically widespread effect extending to the frontal regions. To provide more spatial details about this effect, we also performed a voxel-based control analysis of the above eLORETA solutions (see the Supplementary Materials). In the Nold group, the maximum t values of the differences between DELTA+ and DELTA- were localized in the occipital Brodmann area (BA) 18. In the ADD group, those maximum t values were instead localized more anteriorly, namely in the central BA 6.

Abnormal rsEEG rhythms at delta frequencies in ADD patients

As reported above, the main results of the present rsEEG study showed that in the Nold group, the resting state delta rhythms were linearly and negatively correlated with the alpha rhythms, possibly about the general cortical arousal as revealed by a widespread desynchronization of alpha rhythms. These results lead support to the speculation that those delta rhythms may trigger excitatory processes in the human cerebral cortex even in the quiet wakefulness. In the quiet wakefulness, cortical delta rhythms may be generated by widely distributed neural assemblies, especially distributed in associative regions. Their oscillatory activity at delta frequencies may entrain EEG rhythms at higher frequencies, including the alpha band, and trigger excitatory processes (Nuñez, 1992; Pfurtscheller and Lopes da Silva, 1999).

In this speculative line, earlier studies showed that in healthy adults, resting state delta rhythms were positively correlated with the cortical metabolism as measured by [18F]fluoro-D-glucose (Alper et al., 2006). Furthermore, Dr Erol Başar and colleagues repeatedly showed that (1) cognitive events might induce ample and transient frontal EEG oscillations at delta frequencies with a clear excitatory meaning in healthy adults and (2) those oscillations were deranged in ADD patients (Başar et al., 2001, 2016; Karakaş et al., 2000; Yener et al., 2007, 2008, 2009, 2012).

We also speculate that the mentioned co-fluctuation in the resting delta and alpha rhythms may be related to the regulation of the subject's vigilance in the quiet wakefulness. Indeed, previous rsEEG studies carried out in cognitively intact adults reported that (1) resting state cortical delta rhythms increased and alpha rhythms decreased after sleep deprivation and (2) recovered by the acute administration of pharmacological drugs enhancing subjects' vigilance (Chapotot et al., 2003; Bodenmann et al., 2009; Del Percio et al., 2019; James et al., 2011).

Unfortunately, it is unclear the neurophysiopathological mechanism underlying the abnormalities observed at the resting state delta rhythms in the present ADD patients, namely the derangement of the linear negative correlation with the alpha rhythms. In this regard, it can be speculated that in these patients, the abnormalities of delta rhythms in the quiet wakefulness may be due to an impairment in the functional connectivity among (1) thalamocortical and corticothalamic loops targeting widespread cortical modules in the regulation of quiet vigilance (Steriade and Llinas, 1988; Klimesch, 1999; Babiloni et al., 2010, 2020a, b) and/or (2) cholinergic basal forebrain and cerebral cortex (Wan et al., 2019). Such impairment in that functional connectivity may be provoked by (1) a structural

disconnection of the cerebral cortex provoked by vascular lesions in subcortical white matter bundles (Babiloni et al., 2006d); (2) a reduced regional cerebral blood flow and hypometabolism in bilateral parietal cortex and hippocampi (Galluzzi et al., 2016; Rodriguez et al., 1998, 1999, 2004; Babiloni et al., 2016); (3) AD neuropathology (e.g., low Ab42 and high phospho tau levels); and (4) neurodegeneration of cortical grey matter and hippocampus (Babiloni et al., 2009; 2013, 2016; Galluzzi et al., 2016). Noteworthy, the widespread distribution of rsEEG rhythms at delta frequencies makes it improbable its unique generation from the frontal circuits entraining physiological slow EEG oscillatory activity during non-rapid eye movement sleep (Steriade, 2004).

Methodological remarks

In the interpretation of the present findings, the following methodological limitations should be considered.

The data were collected in all clinical units without a single experimental protocol. As a result, some relevant fluid and neuroimaging biomarkers, clinical and neuropsychological measures, and genetic information were not available in all participants (e.g., just the clinical diagnosis, the MMSE score, and the rsEEG recordings were available in all Nold and ADD participants). In the same vein, the instructions to the Nold and ADD participants and the rsEEG experiments were not harmonized in all clinical units. Moreover, the EEG recordings were performed using different hardware and recording settings (i.e. sampling rates, analogic filters, and reference electrodes). To take into account these differences, a control analysis repeated the analysis of the main hypotheses using “clinical units and EEG systems” as confounding variables. Those variables did not affect the main findings, possibly because (1) those clinical units followed the general guidelines of clinical neurophysiology (Babiloni et al., 2020b) and (2) the recorded rsEEG data were subjected to a central analysis aimed at reducing the variance of the rsEEG variables by the re-referencing to the common average reference, the normalization of rsEEG power density across frequencies and electrodes, and log transformation of the normalized rsEEG power density.

The clinical 10–20 electrode montage (i.e., 19 scalp electrodes) adopted for the rsEEG recordings is sub-optimal for the spatial sampling of rsEEG activity and the source estimation (Liu et al., 2002; Marino et al., 2016). An optimal spatial sampling would have requested > 64 scalp electrodes. Therefore, the current results of the rsEEG source

estimation should be considered as preliminary in the context of an exploratory rsEEG study. In that context, they may be informative for the following reason. RsEEG rhythms show largely dominant low spatial frequency components allowing valid anti-aliasing spatial sampling of instantaneous potential distributions by the mentioned 10-20 montage system using 19 scalp electrodes. This statement is grounded on many previous investigations carried out to study brain “microstates” in humans during the quiet wakefulness, defined as quasi-stable (i.e., few seconds) topographical patterns of rsEEG voltage maps (Lehmann et al., 1987). Indeed, these rsEEG topographic patterns are typically characterized by two very large voltage centroids (i.e. diameter of about ten centimeters), one of the positive potentials and the other of negative potentials, changing position on the scalp over time (Khanna et al., 2015; Michel and Koenig, 2018). These rsEEG topographic patterns, which are generated by widely distributed cortical neural networks revealed by resting-state functional MRI, can be recorded without spatial aliasing by the traditional 10-20 montage system (Khanna et al., 2015; Michel and Koenig, 2018; Riba et al., 2004; Huang et al., 2000; Mulert et al., 2001; Winterer et al., 2001; Veiga et al., 2003; Hata et al., 2016). In this line of reasoning, a Workgroup of International Federation of Clinical Neurophysiology expressed the consensus position that EEG source estimation of rsEEG data collected by the traditional 10-20 montage system can be used for exploratory studies performed on available rsEEG databases relative to neurological and psychiatric patients (Babiloni et al., 2020a). Those exploratory studies may provide informative preliminary results motivating further investments to develop cross-validating prospective rsEEG studies using EEG techniques with higher resolution (i.e., recordings based on 48-256 scalp electrodes, etc.).

Finally, rsEEG recordings referred to a single data acquisition session, thus preventing the analysis of the finding reliability and the relation to patients’ clinical manifestations during the AD progression.

The above limitations and the current results motivate resource investments to develop future prospective, longitudinal, and multi-center studies using (1) diagnostic fluid and neuroimaging biomarkers of AD; (2) a detailed evaluation of the patient’s cognitive status; (3) harmonized EEG hardware systems and protocols; (4) a higher number of exploring scalp electrodes for enhanced rsEEG source estimates; (5) a finer individual analysis of delta and alpha frequencies of rsEEG rhythms; and (6) the use of new techniques testing a significant relationship between the phase of delta rhythms and the

amplitude in alpha rhythms as a neurophysiological cortical mechanism regulating cortical arousal in quiet wakefulness.

Conclusions

Here we hypothesize that in Nold seniors, transient increases in resting state delta rhythms may enhance global cortical arousal as revealed by alpha1 rhythms and (2) this relationship may derange in ADD. As main findings, the Nold group showed a linear and negative correlation across individual between the global delta and alpha1-2 power density. In the ADD group, such a correlation was just marginal. However, both the Nold and ADD groups similarly showed that the global alpha1 power density was lower (pointing to greater cortical arousal) in the rsEEG epochs showing the highest over the lowest global delta power density. An explorative control analysis by eLORETA source estimation showed that these effects were distributed in the posterior cortical regions in both Nold and ADD groups, with a frontal extension in the ADD group.

These results suggest that AD may mitigate the cross-band neurophysiological mechanism inter-relating delta and alpha rhythms in the quiet wakefulness, with potential effects on the regulation of patients' vigilance.

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Table legends

Table 1. Mean values ($\pm$ standard error of the mean, SE) of the demographic and clinical data as well as the results of their statistical comparisons ($p < 0.05$) in the groups of healthy elderly participants (Nold, $N = 100$) and patients with dementia due to Alzheimer's disease (ADD, $N = 100$). Legend: MMSE = Mini Mental State Evaluation; M/F = males/females; n.s. = not significant ($p > 0.05$).

Table 2. Brodmann areas (BAs) corresponding to the regions of interest (ROIs) where we averaged the solutions of the exact low-resolution brain electromagnetic tomography (eLORETA) freeware to estimate cortical sources of eyes-closed resting state electroencephalographic (rsEEG) rhythms recorded in the quiet wakefulness.

Figure legends

Figure 1. Scatterplots showing the global scalp normalized delta (2-4 Hz) and alpha1 (8-10.5 Hz) power density in the normal elderly (Nold) seniors and patients with Alzheimer's disease dementia (ADD), derived from resting state eyes-closed electroencephalographic (rsEEG) recordings. Pearson test evaluated the hypothesis of a correlation between these delta (2-4 Hz) and alpha1 (8-10.5 Hz) power density values in the Nold and ADD groups, considered separately ($p < 0.05$ corrected). The r and p values of the correlation analysis are reported in the diagrams.

Figure 2. (Top): Global scalp normalized rsEEG power density values (mean across subjects, log 10 transformed) computed in the Nold group in relation to a statistical ANOVA interaction between the factors Delta (50% of the rsEEG epochs showing the highest delta power density, DELTA+, and 50% of the rsEEG epochs showing the smallest delta power density, DELTA-) and Band (delta, theta, alpha1, alpha2, beta1, beta2, and gamma).

(Bottom): Global scalp normalized rsEEG power density values (mean across subjects, log 10 transformed) computed in the ADD group in relation to a statistical ANOVA interaction between the factors Delta (DELTA+ and DELTA-) and Band (delta, theta, alpha1, alpha2, beta1, beta2, and gamma). Legend: the asterisks indicate the frequency bands in which the global scalp normalized rsEEG power density values presented statistically significant effects in line with the pattern "DELTA+ $\neq$ DELTA-" ($p < 0.05$ corrected).

Figure 3. Scatterplots illustrating the results of the control analysis using Pearson test ($p < 0.05$ corrected) to evaluate whether the linear correlation between the global scalp normalized delta and alpha1 power density (log 10 transformed) may be higher in the rsEEG epochs with the lowest (i.e., DELTA-) than the highest (DELTA+) global scalp delta power density computed in the Nold and ADD groups, considered separately.

Figure 4. Global scalp normalized power density (mean across subjects, log 10 transformed) computed from rsEEG rhythms in relation to a statistical ANOVA interaction between the factors Group (healthy older participants, Nold, and ADD) and Band (delta, theta, alpha1, alpha2, beta1, beta2, and gamma). Legend: the asterisks indicate the frequency bands in which the global scalp normalized rsEEG power density values statistically significant effects such as a pattern "Nold $\neq$ ADD" ($p < 0.05$ corrected).

Figure 5. Scatterplots showing the relationship between the global scalp normalized delta power density (log 10 transformed) and the global scalp normalized alpha1 and alpha2 power density computed on individual basis in the Nold and ADD groups (see Materials and Methods). Pearson test evaluated that relationship ($p < 0.05$ corrected). The r and p values are reported in the diagrams.

Figure 6. (Top): Regional normalized alpha1 eLORETA current density (mean across subjects, log 10 transformed) estimated from the rsEEG data recorded in the Nold group. The diagram represents the effects of a statistical ANOVA interaction between the factors Delta (DELTA+ and DELTA-) and Region of Interest (ROI; frontal, central, parietal, occipital, temporal, and limbic). (Bottom): Regional normalized alpha1 eLORETA current density (mean across subjects, log 10 transformed) estimated from the rsEEG data recorded in the ADD group. The diagram represents the effects of a statistical ANOVA interaction between the factors Delta (DELTA+ and DELTA-) and ROI (frontal, central, parietal,

occipital, temporal, and limbic). Legend: the asterisks indicate the ROIs in which the regional normalized alpha1 eLORETA current density presented statistically significant effects in line with the pattern “DELTA+ $\neq$ DELTA-” (Duncan post-hoc test, $p < 0.05$ corrected).

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Table 1

	Nold	ADD	Statistical analysis
N	100	100	
Age	69.0 ± 0.6SE	69.1 ± 0.7 SE	t-test: n.s.
Gender (M/F)	38/62	38/62	Fisher test: n.s.
Education	9.7 ± 0.4 SE	9.6 ± 0.4 SE	t-test: n.s.
MMSE	28.8 ± 0.1 SE	19.2 ± 0.3 SE	Mann-Whitney U test: p < 0.000001

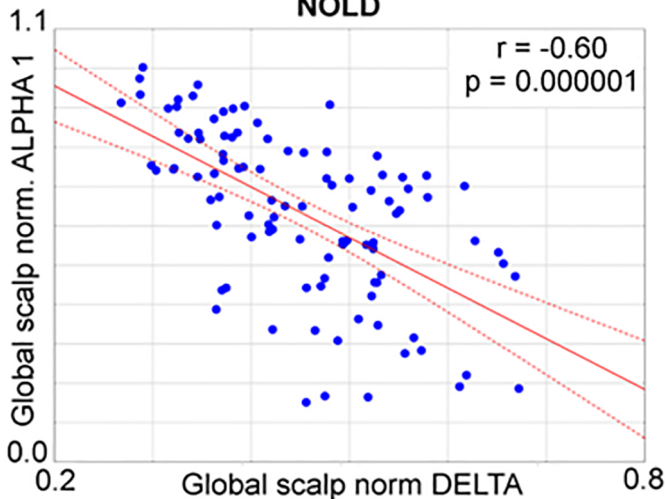
Table 2

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
Frontal	8, 9, 10, 11, 44, 45, 46, 47

- Resting EEG rhythms differ in Alzheimer's and healthy seniors (group level)
- Alzheimers' seniors show abnormally high delta and low alpha rhythms
- Delta and alpha power densities are negatively correlated in healthy seniors
- Above correlation is mitigated by Alzheimer's disease (dementia)

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SCALP ALPHA 1 AND DELTA POWER DENSITY NOLD



ADD

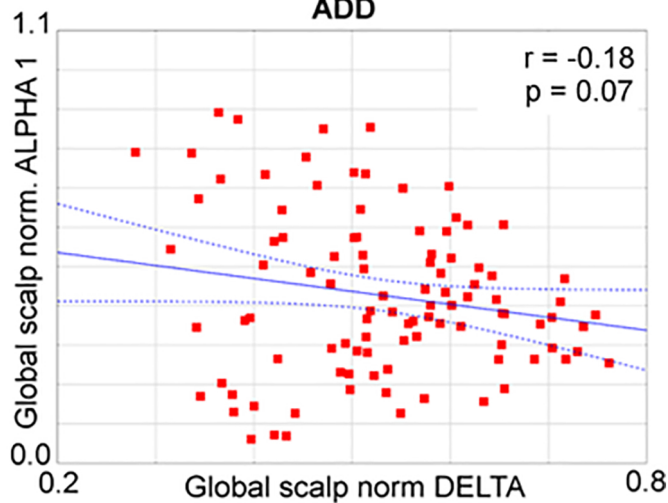


Figure 1

STATISTICAL ANOVA INTERACTION AMONG DELTA AND BAND

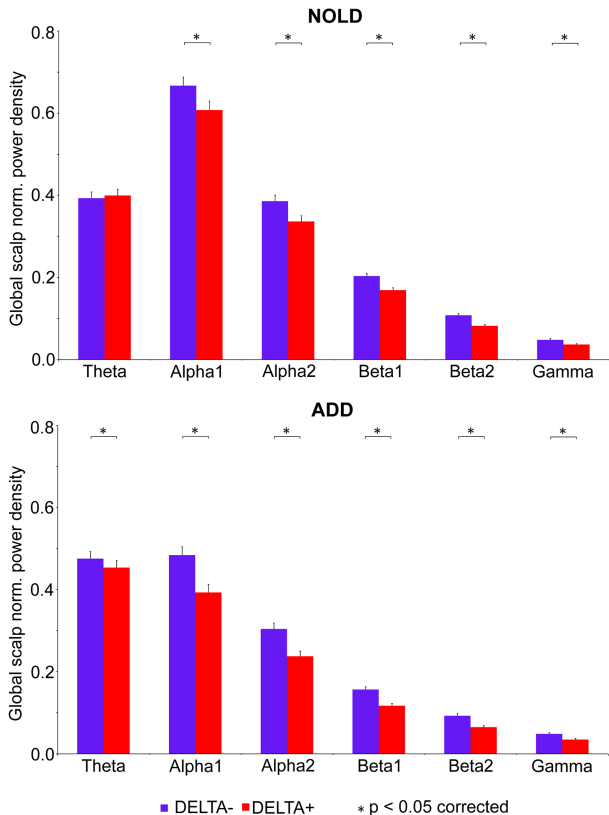


Figure 2

SCALP ALPHA 1 AND DELTA POWER DENSITY

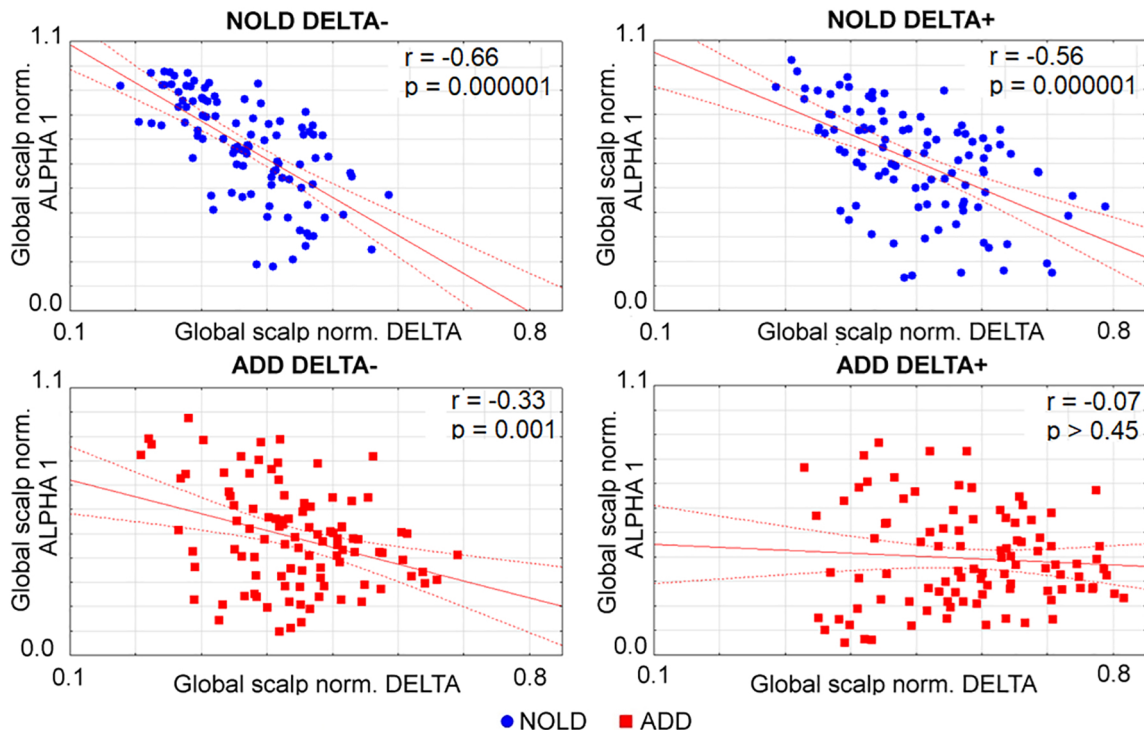


Figure 3

STATISTICAL ANOVA INTERACTION BETWEEN GROUP AND BAND

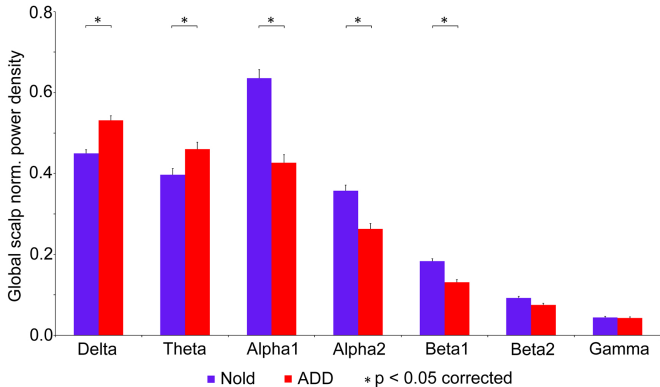


Figure 4

SCALP INDIVIDUAL ALPHA AND FIXED DELTA POWER DENSITY

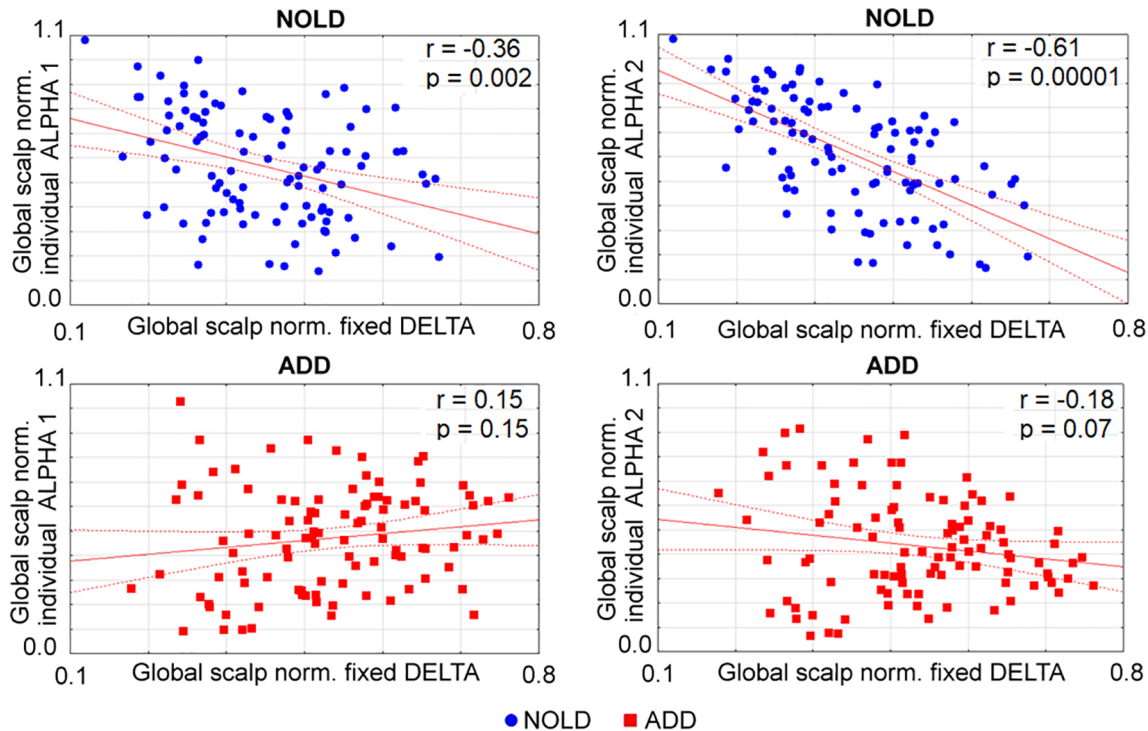


Figure 5

STATISTICAL ANOVA INTERACTION AMONG DELTA AND BAND

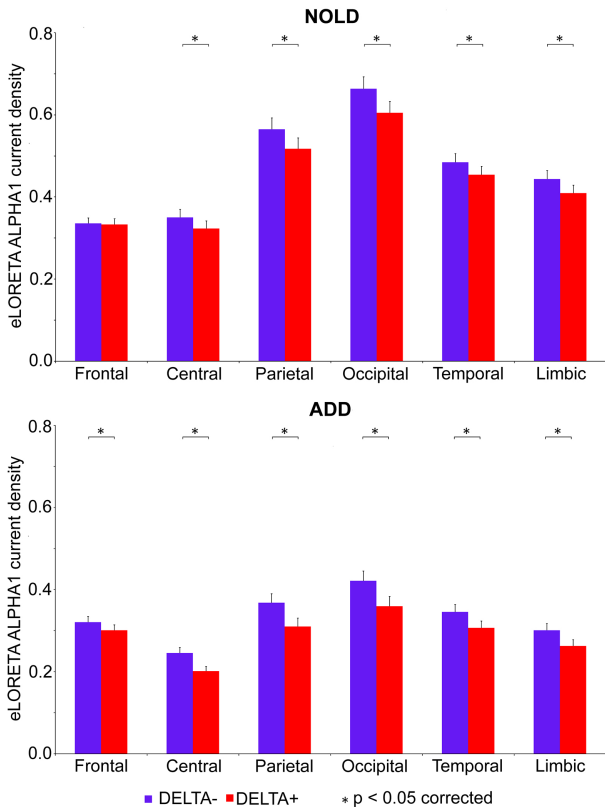


Figure 6