

07/25/2023

**THE UNDERESTIMATED UTILITY OF RESTING-STATE GAMMA
OSCILLATIONS: BOTH INCREASE AND DECREASE OF GAMMA
DISCRIMINATES EARLY-ONSET ALZHEIMER'S DISEASE PATIENTS FROM
HEALTHY INDIVIDUALS**

STUDY PROTOCOL:

- **EEG Recording and Analysis**

Instructions Prior to Experiment and EEG Recording Environment

- Participants were asked not to consume any caffeine prior to experiments.
- EEG was recorded in a sound-attenuated and electrically shielded room.

EEG Recording Setup

- EEG was amplified using a BrainAmp 32-channel DC amplifier
- EEG was bandpass-filtered between 0.03-70 Hz and digitized at a sampling rate of 500 Hz (Brain Products GmbH; Gilching, Germany).
- EEG was recorded from 30 Ag/AgCl electrodes (Figure 1).
- Electrodes were positioned on an elastic cap according to the international 10-20 system (EasyCap; Brain Products GmbH; Gilching, Germany).
- All electrodes were referenced to linked earlobes (A1+A2).
- Electrooculograms (EOG) were recorded from two additional electrodes.
- Electrode impedances were kept below 10 k Ω .
- EEG recordings were performed during morning hours (approximately between 09.00 to 10.00 am)
- EEG recordings lasted 8 minutes (4 minutes: eyes open & 4 minutes: eyes closed).

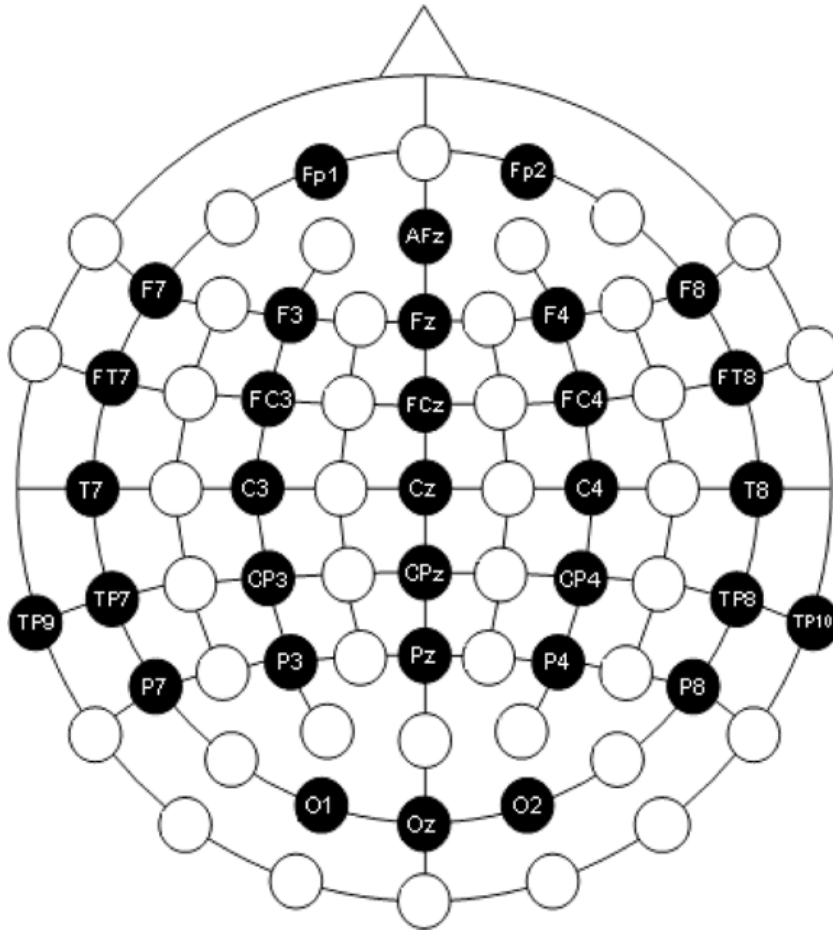


Figure 1. EasyCap layout for 30 Electrodes (EasyCap; Brain Products GmbH; Gilching, Germany)

EEG Data Preprocessing

Brain Vision Analyzer v.2.2 (Brain Products GmbH; Gilching, Germany) program was used for offline data analysis. EEG preprocessing was employed as below;

- The continuous EEG data were resampled to a 256 Hz sampling rate.
- A band-pass filter at 0.1 with a 12 dB/octave slope and a 50 Hz notch filter was applied.
- Vertical and horizontal ocular artifacts were corrected with independent component analysis (ICA) with the Infomax algorithm using default parameters.
- Four minutes eyes-closed recordings were segmented into six-second epochs.

- Artifacts that still contaminated the EEG data (i.e., muscle artifacts and drowsiness) were semi-automatically rejected based on maximum voltage steps of 50 $\mu\text{V}/\text{ms}$, amplitudes exceeding $\pm 70 \mu\text{V}$, and activity below 0.5 μV parameters.

Power Spectral Analysis

A Fast Fourier Transform (FFT) was applied with

- Maximum resolution power,
- Non-complex output on full-spectrum,
- 10% Hanning window function

Coherence Analysis

- The Laplacian Current Source Density transformation with an order of splines 4, the maximal degree of Legendre polynomials 10 and 1×10^{-5} Lambda was applied.
- The FFT with maximum resolution voltage, no window function, and complex output on the half spectrum was computed.
- The imaginary part of coherency was used to evaluate connectivity between hemispheres, within hemispheres, and midline connectivity.

● **MRI Acquisition**

- Philips Achieva 1.5 Tesla scanner was used.
- Scans were performed according to the Alzheimer's disease Neuroimaging Initiative (ADNI, www.adni.loni.usc.edu).
- The pre-processing of images was performed with the SPM12 software package.
- The gray matter volumes and cortical thickness were obtained from the 3D-T1 weighted TFE sequence (TR: 9ms, TE: 4ms, FOV: 240mm, matrix: 256, slice thickness: 1mm, NSA: 1).
- Mean gray matter volumes (GMVs) inside ROIs were estimated using the LPBA40.
- The total intracranial volume (TIV) for each participant is calculated and exported. GMVs were normalized according to each individual's TIV to remove the effect of different brain sizes.

STATISTICAL ANALYSIS PLAN (SAP)

- **For EEG Analysis:**

The following EEG measures were analyzed using separate repeated-measures ANOVA including group as a between-group factor and different within-subject factors for;

(1) the EEG power [*anterior-posterior distribution* (frontal, central, parietal, and occipital) and *laterality* (left, midline, and right)];

(2) EEG intra-hemispheric ICoh measures [*region* (14-levels) and *hemisphere* (left and right)];

(3) midline-to-lateral ICoh measures [*midline region* (4-levels), *lateral region* (8-levels) and *hemisphere* (2-levels)];

(4) midline-to-midline ICoh measures [*midline region* (3-levels)];

(5) inter-hemispheric ICoh measures [*region* (10-levels)].

All analyses were performed for total gamma and sub-gamma frequency bands.

When Mauchly's test indicated that the assumption of sphericity had been violated, the Greenhouse-Geisser correction was applied where $\varepsilon < 0.75$, and the Huynh-Feldt correction was applied where $\varepsilon > 0.75$.

- **For EEG Analysis:**

GMV measures were analyzed with univariate ANOVA. Age and gender variables were included in the analysis as covariates. Bonferroni correction was applied for 54 GMV ROIs. The significance level was set to $P < 0.0009$.

- **For Correlations Analysis:**

Correlations between CSF, NPT, EEG, and MRI parameters were analyzed with Pearson correlation analysis. In order to minimize the number of false positives in correlation analysis, a Benjamin-Hochberg false discovery rate (FDR) was used to control the type-I error.

- **For Discriminant Analysis:**

The discriminant analysis was performed including the group as the dependent variable and a total of four independent variables (three electrode pairs from coherence and one electrode from power analysis) to the model. The analysis was performed with the enter method.