Insula functional connectivity at EEG resting-state as a biomarker linked to pain processing and severity of clinical symptoms in fibromyalgia

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Abstract

Brain areas and oscillations have been pointed out as markers of chronic pain. We used electroencephalography (EEG) to compare the linear and non-linear connectivity of regions of interest (ROIs) related to the pain matrix in people with fibromyalgia (FM) and healthy controls (HC). Sixty-four women (49 FM and 15 HC) volunteered to participate in a resting-state EEG session with eyes open (EO) and eyes closed (EC), as well as clinical, psychological, and serum brain-derived neurotrophic factor (BDNF). The connectivity of 10 ROIs was computed for 7 different EEG frequencies. In EC conditions, FM exhibits decreased non-linear connectivity in the beta-2 band between the right-insula and left mPFC (F=-4.09, p = .035). Considering the differences between EO and EC, FM present increased linear connectivity between the left insula and right thalamus in the beta-3 band (F = 3.46, *p = .009*) and increased non-linear connectivity between the right insula and left medial prefrontal cortex (mPFC) in the beta-2 band (F = 3.78, *p = .025*). These results mainly indicate the insula role in different circuits in FM and highlight the beta oscillations activity. Insular brain connectivity at rest could be an objective biomarker of how pain is processed, and it is related to the severity of clinical symptoms in fibromyalgia.

1. Introduction

Fibromyalgia (FM) is a nociplastic pain syndrome characterized by widespread musculoskeletal chronic pain, fatigue, non-restorative sleep, cognitive impairment, and mood disturbances [1]. It is the third most common musculoskeletal condition, increasing with age and being more common in women [2]. FM is a primary chronic pain syndrome that is caused by increased excitability and synaptic efficiency or decreased inhibition of medullary and supramedullary nociceptive pathway neurons [3].

Functional magnetic resonance imaging (fMRI) studies on humans and animals have shown that many regions of the brain have their blood flow modulated when a painful stimulus is present, depending on how the pain feels and how it is perceived. Because cognitive and emotional brain areas are involved in chronic pain, it is felt through different brain networks than acute pain. The pain matrix is made up of the thalamic nuclei (TH), the primary and secondary somatosensory cortices (SI and SII), the insula, the anterior cingulate (ACC), and the prefrontal cortices (PFC), all of which are more active after pain stimulation [4].

Peyron and Fauchon (2019) [5] say that the pain matrix is a complex neural network that includes many functions, such as motor withdrawal from pain, attention, anticipation, memory, habituation, and other processes that make up the pain experience. The insula is a cortical structure below the frontal, temporal, and parietal lobes, deep in the lateral sulcus, where different kinds of information are brought together. The affective and motivational parts of pain are controlled by the anterior insula, and the sensory and discriminative parts are controlled by the posterior insula [6]. The way the insula and ACC are set up acts as a salient stimulus switch, drawing attention to the prefrontal cortex (PFC), which is connected to the default mode network (DMN) and the executive control network (CEN). A previous study found that people with chronic pain might have changes in how their brains connect structurally and functionally in the pain matrix areas [7].

Chronic pain conditions demonstrate alterations in the cortico-limbic and cortico-cortical areas. The medial PFC plays an important role in how we deal with emotional pain. It also controls pain from top-down pathways by sending direct projections to the periaqueductal gray (PAG), which is a key part of the descending pain modulatory system (DPMS) [8]. According to changes in the connectivity of pain-related networks in FM during rest, chronic pain alters how the brain functions [9]. During the resting state, FM patients had more connections between the DMN and the insula than healthy controls [10]. The DMN is linked to the severity of pain in FM because salient and unpleasant chronic pain experiences make people more alert [11]. Ong, Stohler and Herr (2019) [12] found that changes in the structure, activity, and connections of the PFC are linked to both acute and chronic pain. Also, left PFC activation measured by functional near-infrared spectroscopy (fNIRS) after thermal stimulation was a sensitive sign in FM patients with more severe clinical symptoms [13]. Neuroplasticity is a key part of the central nervous system (CNS), and the brain-derived neurotrophic factor (BDNF) is a marker of synaptic plasticity in the brain [14]. Also, the Val/Met polymorphism is linked to more functional connections between the prefrontal cortex (PFC) and the motor cortex (MC), while the Val/Val polymorphism is linked to less functional connections between these two areas, less active engagement of the descending pain modulatory system (DPMS), and a greater effect of fibromyalgia symptoms on quality of life [15]. In another study, FM patients using opioids showed less variation in peak amplitudes of electroencephalography oscillations during EO-EC than non-opioid users in the bands of central theta, central beta, and parietal beta. This smaller oscillation in the parietal delta band of cortical activity was linked to pain-related disability in a bad way. So, these electrophysiological measures show that the severity of FM is linked to worse cortical processing [16]. So, brain electrical activity oscillation might be a good and sensitive way to figure out how neuroplasticity affects the severity of a disease.

The eyes-open (EO) and eyes-closed (EC) states measured by the EEG resting-state paradigm are linked to changes in EEG that show how the brain's activity changes in response to visual stimuli. Barry *et al.* (2007) [17] support the idea that the baseline state of arousal depends on the organism's current physiological level, while the activation process depends on how much energy is used to respond to a stimulus. Comparing the functional connectivity and brain activity of FM and HC in a resting state with EEG shows that pain inhibition happens when different brain areas are activated and connected through brain networks like the DMN and salience network (SN) [18].

Based on what was said above, we think that FM patients have changes in the way brain activity, as shown by cerebral frequency bands, and the salience network, which is involved in processing pain, work together. So, the study's goal was to see if the connectivity of ROI involved in the pain matrix could be different between FM patients and healthy controls (HC) based on EEG frequency band variations between eyes open (EO) and eyes closed (EC) conditions. In FM, using the same method, we looked to see if there was a link between the brain oscillation of the ROIs and clinical, psychological, and serum brain-derived neurotrophic factor (BDNF).

2. Methods

2.1 Study Design and settings

The Research Ethics Committee at the Hospital de Clinicas de Porto Alegre (HCPA) approved the study under the registration number (2020 – 0369) according the international ethical standards based on the Declaration of Helsinki. The study was conducted in accordance with the relevant guidelines and regulations. All participants provided written informed consent before participating in this study.

2.2 Participants, Recruitment, Inclusion, and Exclusion Criteria

Participants were women aged 30 to 65 diagnosed with FM, according to the American College of Rheumatology (ACR) 2016 [1]. They needed to be literate and report a score of 6 or higher on the Numerical Pain Scale (NPS 0–10) most of the time in the last three months. A team with extensive experience in pain management confirmed the diagnosis. They were not included in the study if they had used alcohol or drugs in the last six months, were pregnant, had a neurological disease, or had a history of head trauma or neurosurgery. Besides, if they had decompensated systemic diseases, chronic inflammatory diseases, uncompensated hypothyroidism, another metabolic disease, or were getting treatment for cancer,

In this study, screening was done on 133 FM participants who were eligible to take part. However, 67 of them did not meet the inclusion criteria for different reasons, such as living far away from the research center, having trouble getting around on public transportation, being unemployed, etc. Some of the screened participants did not meet the diagnostic criteria for FM. Besides, they were excluded if they met such diagnosis criteria: their pain levels were lower than 6 (NPS 0–10) or they had another uncompensated clinical disease (rheumatoid arthritis, lupus, hypothyroidism, etc.). So, 66 FM were included in the study, but 17 of them were excluded due to low-quality EEG signals. So, at the end, 49 subjects were included in the analysis.

The control (HC) subjects were literate women aged 30 to 65. They were recruited from the local community through social media. They had to take a phone test to make sure they weren't sick or on medicine. In the HC, 33 participants were screened, and 16 were included. Seventeen people were taken out of the study because their Beck Depression Inventory-II (BDI-II) score was higher than 13 [19] or because they regularly took painkillers, antidepressants, anticonvulsants, anxiety-reducers, hypnotics, etc. Also, one subject was excluded from the EEG preprocessing data due to low-quality signals. Thus, the final sample of HC comprised 15 participants.

The final sample comprised 64 participants (49 FM and 15 HC). Their demographic and clinical measures are presented in Table 1. We found significant differences in years of formal study and age between the FM and HC groups.

Table 1.

Table 1

Demographic and clinical characteristics of the study sample. Values are presented as mean (SD) or relative frequencies (RF) and percentage (n = 64). P-values for continuous variables (demographic, biochemical, mood, pain-related, and sleep quality measures) are based on a parametric t-test. Categorical variables (clinical comorbidity, psychiatric disorders, and medication use) are based on Fisher's exact test.

	FM GROUP (N = 49)		**HC GROUP (N = 15)**		
	Mean (SD) or RF (%)	**Median (IQ 25–75)**	**Mean (SD) or RF (%)**	**Median (IQ 25–75)**	***p*-values**
Demographic measures					
Age (years)	48.06 (9.83)	47.00 (40.50, 56.00)	41.47 (8.42)	40.00 (34.00,50.00)	**< 0.05**
Years of formal study	12.07 (4.00)	11.00 (9.25, 15.50)	15.73 (5.06)	17.00 (11.00, 20.00)	**< 0.05**
American College of Rheumatology (ACR) diagnosis tool	22.78 (3.74)	23 (19.50, 25.00)	-	-	-
Employed (Yes/No)%	29/20 (59.2%)	-	12/3 (80.0%)	-	0.220
Smoking* (Yes/No)%	12/37 (24.5%)	-	0/15 (0.0%)	-	0.054
Drinking* (Yes/No)%	24/25 (49.0%)	-	10/15 (66.7%)	-	0.225
Clinical comorbidity					
HAS (Yes/No)	15/34 (30.0%)	-	3/12 (20.0%)	-	-
Cardiac Disease (Yes/No)	2/47 (4.1%)	-	1/14 (6.7%)	-	-
Diabetes Disease (Yes/No)	4/45 (8.2%)	-	0/15 (0.0%)	-	-
Hypothyroidism (Yes/No)	7/42 (14.3%)	-	0/15 (0.0%)	-	-
Asthma (Yes/No)	12/37 (24.5%)	-	0/15 (0.0%)	-	-
Epilepsy (Yes/No)	0/49 (0.0%)	-	0/15 (0.0%)	-	-
Renal Insufficiency (Yes/No)	1/48 (2.0%)	-	0/15 (0.0%)	-	-
Biochemical measures					

	FM GROUP (N = 49)		HC GROUP (N = 15)		
	Mean (SD) or RF (%)	Median (IQ 25–75)	Mean (SD) or RF (%)	Median (IQ 25–75)	*p*-values
Demographic measures					
Serum BDNF (pg/ml)	45.25 (32.87)	31.64 (21.16, 68.65)	-	-	-
Mood, pain and sleep quality measures					
Beck Depression Inventory (BDI-II)	24.82 (11.01)	24.00 (14.50, 34.50)	-	-	-
Brazilian Portuguese Central Sensitization Inventory (BP-CSI)	62.90 (13.88)	65.00 (52.00, 75.50)	-	-	-
Brazilian Portuguese Pain Catastrophizing Scale	35.57 (10.88)	36.00 (29.00, 44.00)	-	-	-
Pittsburgh Sleep Quality Index (total score)	12.22 (3.53)	13.00 (9.50, 14.00)	-	-	-
Fibromyalgia Impact Questionnaire (FIQ) (total score)	67.41 (16.66)	71.57 (62.17, 78.40)	-	-	-
Psychiatric disorder					
Major Depressive Disorder - MDD (current) (Yes/No)	24/25 (49.0%)	-	-	-	-
Generalized Anxiety Disorder[a] – GAD (Yes/No)	10/39 (20.4%)	-	-	-	-
Medication use					
Antidepressant (Yes/No)	29/20 (59.2%)	-	-	-	-
Anticonvulsivant (Yes/No)	11/38 (22.4%)	-	-	-	-
Benzodiazepines (Yes/No)	7/42 (14.3%)	-	-	-	-
Opioid analgesic (Yes/No)	13/36 (26.5%)	-	-	-	-
Non-opioid analgesic (Yes/No)	45/4 (91.8%)	-	-	-	-

	FM GROUP (N = 49)		HC GROUP (N = 15)		
	Mean (SD) or RF (%)	Median (IQ 25–75)	Mean (SD) or RF (%)	Median (IQ 25–75)	*p*-values
Demographic measures					
HAS[a] (Yes/No)	12/37 (24.5%)	-	-	-	-

2.3 Instruments and Assessment of Outcomes

Dependent and independent variables.

The dependent variables (outcome) were the lagged-linear connectivity (LLC)/coherence and the lagged nonlinear connectivity (LNLC)/phase synchronization between regions of interest (ROIs) of the pain matrix assessed by delta, theta, alpha-1, alpha-2, beta-1, beta-2, and beta-3 EEG frequencies at resting state. Secondary outcomes were the source localization analysis of the distinct 7 frequency bands in their resting state. Other variables of interest were evaluated in FM subjects, such as the intensity of pain, the BDNF serum levels, pain catastrophizing, depressive symptoms, sleep quality, sociodemographic characteristics, clinical and psychiatric chronic diseases, and psychotropic and analgesic medications. The sequence of assessments is presented in Fig. 1.

2.4 Assessment of primary outcome

1. *EEG recording*: The assessments were conducted in a quiet room with subjects sitting in comfortable armchair. The electroencephalography was recorded using 18 scalp sites according to the 10–20 system. FP1, FP2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, Oz, and the left ear (EXT), with reference to the right ear (CMS/DRL). The EEG system used was the ENOBIO 20, made by Neuroelectrics in Barcelona, Spain. It has a cap with 1.75 cm2 of gel electrodes in a circle. Impedance was 5 kΩ for all electrodes, with high dynamic resolution (24 bits, 0.05 uV) and a sampling rate of 500 Hz. A line noise filter (60-Hz) was applied to remove main line artifacts from the EEG data.
2. *Resting-state paradigm*: The resting-state EGG was collected for 8 minutes total, with 2 minutes switched between EC and EO conditions. Participants were instructed to remain awake, relaxed, and thinking-free. During the EO condition, the people who took part were told to keep their eyes on a small cross in front of them. The EC condition is the level of arousal at rest, and the EO condition is the level of arousal at activation. The difference between EO and EC represents the activation process [17].

2.5 Preprocessing and functional connectivity analysis

The EEG data were cleaned up using the open-source toolbox EEGLAB 14.1 [20], which ran in the MATLAB environment (The MathWorks Inc., Natick, Massachusetts, United States). Visual inspection was performed for artifact detection, and segments of bad channels were removed, if necessary. Continuous EEG data was

band-pass filtered using a simple FIR filter with cutoff frequencies of 0.5–40 Hz, resampled to 250 Hz, and split into 4.096 s epochs [21].

Rejection thresholds were determined according to artifact attributes. To get rid of eye blinks and other quick movements from non-filtered continuous EEG, 50μV thresholds were set for FP1 and FP2 electrodes and 100μV thresholds for other electrodes. To eliminate artifacts associated with slow head or body movements, 50μV thresholds were used for slow waves (0–1 Hz band), while 30μV thresholds were used for fast waves (20–35 Hz band). Epochs containing artifacts were automatically excluded from the analysis [22]. To compute the source localization and brain connectivity, the minimum threshold was fixed at 40 seconds for each resting-state condition (EO, EC, and the difference between EO and EC) [21]. Subjects or conditions below this threshold were rejected for the analysis.

Standardized low-resolution brain electromagnetic tomography (sLORETA) was used to figure out the current density distribution from images of electric neuronal activity. This functional imaging method determines the topographic sources based on images of standardized current density distributions with high spatial accuracy and low resolution of brain function according to the principles of linearity and superposition [23]. The LORETA-Key software is freely available at https://www.uzh.ch/keyinst/NewLORETA/Software/Software.htm.

EEG electrode coordinates employed by the software are based on the MRI anatomical template from the Montreal Neurological Institute (MNI152) which slices and classifies the neocortical volume (limited to the gray matter) in 6239 voxels of dimension $5mm^3$ [24].

Functional connectivity measures can be estimated using the sLORETA algorithms, which compute the linear dependence (coherence) and nonlinear dependence (phase synchronization) of the electric neuronal activity from several brain regions [25].

However, dependence measures are highly contaminated with instantaneous, non-physiological effects, due to volume conduction and the low spatial resolution of EEG. The development of a new measure of coherence that considers only lagged connectivity between several brain locations to verify the existence of distributed cortical networks.

Lagged-linear connectivity (LLC) expresses the coherence measured by the corrected standardized covariance of scalp electric potentials, extracting the instantaneous linear dependence, whereas lagged nonlinear connectivity (LNLC) shows the connection between locations with a corrected phase-synchronization value over time [25] [26].

The dependence measures were introduced for discrete frequency components, but can be applied to any frequency band, defined as a set of discrete frequencies. Therefore, sLORETA functional images of spectral density LLC and LNCL were computed to follow the frequency band: delta (1 ± 3.5 Hz), theta (4 ± 7.5 Hz), alpha-1 (8 ± 10 Hz), alpha-2 (10-12Hz), beta-1 (13 ± 18 Hz), beta-2 (18.5 ± 21 Hz) and beta-3 (21.5 ± 30 Hz).

A voxel-wise approach was implemented to generate the regions of interest (ROIs), MNI coordinates of the areas under the electrode were determined by sLORETA. The left and right primary somatosensory cortex

(BA01), the left and right insular cortex (BA13), the left and right anterior cingulate cortex (BA24), the left and right medial prefrontal cortex (BA09-BA10), and the left and right thalamus were among the ten ROIs that were included, according to previous studies on the pain matrix. The coordinates are shown in Table 2 [9].

Table 2.

Table 2
Montreal Neurological Institute (MNI) coordinates of regions of interest (ROIs). ACC stands for anterior cingulate cortex, INS for insula, mPFC for medial prefrontal cortex, S1 for primary somatosensory, and TH for thalamus.

Seed	MNI coordinates			Brodmann Area
	x	*y*	*z*	
Left S1	-48	-24	52	BA01
Right S1	52	-16	44	
Left INS	-48	12	-2	BA13
Right INS	36	6	6	
Left ACC	-2	8	30	BA24
Right ACC	1	8	30	
Left mPFC	-2	46	-16	BA09 and
Right mPFC	2	46	-16	BA10
Left TH	-10	-22	6	
Right TH	-10	-19	6	

Source localization analysis in all electrode coordinates was performed. Some artifact-free EEG intervals were turned into ASCII files and then put into the sLORETA software. The segmented EEG intervals recorded in resting-state were analyzed by means of a fast Fourier transform algorithm, with 2 seconds of intervals according to the following frequencies: delta (2 ± 3.5 Hz), theta (4 ± 7.5 Hz), alpha-1 (810 Hz), alpha-2 (10-12Hz), beta-1 (13 ± 18 Hz), beta-2 (18.5 ± 21 Hz), and beta-3 (21.5 ± 30 Hz) [27].

Significant source localization differences of spectral power density between FM and HC was performed, comparing the differences between EO and EC conditions (EO-EC), and into EO and EC solely. sLORETA statistical contrast maps were calculated for the 7 frequency bands by multiple voxel-by-voxel comparisons in a log-F-ratio. Cortical voxels that presented significant differences were identified by statistical nonparametric mapping (SnPM) according to a randomization test with 5000 permutations.

Using *t*-tests, multiple comparisons were taken into account when making the maps of connectivity nodes in LLC/coherence and LNLC/phase synchronization for each frequency band across the 10 ROIs. SnPM was used, and the significant threshold difference was based on a randomization test with 5000 permutations.

2.6 Assessment of clinical, psychological, and biochemical variables.

The tools used to measure psychological and clinical measures were validated in the Brazilian population, and the assessment was done by a psychiatrist and trained psychologists.

a) Sociodemographic Questionnaire contains information related to age, years of study, questions about clinical diagnoses, health problems (self-reported), and use of medication.

b) Mini-International Neuropsychiatric Interview (MINI) is a short (15–30 min) structured diagnostic interview aimed to screen for DSM-IV and ICD-10 diagnoses. In the present study, we reported information related to major depressive and manic episodes, panic disorder, social phobia, obsessive-compulsive disorder, post-traumatic stress disorder, and generalized anxiety disorder [28].

c) The Beck Depression Inventory (BDI) is a self-report questionnaire that evaluates depressive symptom severity [19].

d) The Brazilian Portuguese translation of the Pain Catastrophizing Scale (BP-PCS) was used to assess pain's emotional dimension and measure how patients perceive it. It is divided into three domains: magnification, helplessness, and rumination, and the questions are made in order to ask the patient’s feelings and thoughts when they are in pain [29].

e) The central sensitization inventory (CSI) is a tool that identifies key symptoms related to central sensitization processes by quantifying the severity of these symptoms. Part A is a 25-item self-report questionnaire designed to assess health-related symptoms, and Part B (not rated) is designed to determine if one or more specific disorders [30].

f) The Fibromyalgia Impact Questionnaire (FIQ) was applied to assess how quality of life is negatively influenced by FM clinical condition. The questionnaire is composed by 10 items with scores 0 to 10. Therefore, the maximum score is 100. Greater scores denote more impact of symptoms due to FM on quality of life [31].

g) The sleep quality was measured by Pittsburgh Sleep Quality Inventory (PSQI). This self-reported instrument is used to assess the sleep quality and sleep disturbances, that were present over a month, through questions about how long it takes to fall asleep, how long people sleep, how they feel about the quality of their sleep, if they take sleeping pills, if they have trouble sleeping during the day, how well they sleep, and if they have problems sleeping. The sum of these items classifies the subjects into two groups: good sleepers and poor sleepers [32].

h) Enzyme-linked immunosorbent assay (ELISA) monoclonal antibodies specific for BDNF were used to measure the blood levels of BDNF (R&D Systems, MN, USA; ChemiKine BDNF Sandwich ELISA kit, CYT306; Chemicon/Millipore, Billerica, MA, USA). The inter-essay variance was performed using two plates per kit on two days during the same week. All procedures adhered to the manufacturer's recommendations, being 7.8

pg/ml the lowest detection limit for BDNF. ELISA was measured by optical density at 450 nm (Promega, WI, USA; GloMax®-Multi Microplate Reader). Multiplexing assay measurements were conducted in the Bio-Plex®-200 instrument (Bio-Rad). Total protein was assessed using bovine serum albumin following the Bradford method.

2.7 Statistical Analysis

Descriptive statistics were used to summarize the main socio-demographic features of the sample. Data distribution was performed using the Shapiro-Wilk test. To compare continuous variables between groups, t-tests for independent samples were used. The chi-squared and Fisher's exact tests were used to compare groups for categorical variables.

For the FM patients, a linear regression was done between ROI connectivity (the dependent variable) and FIQ, CSI, BDI, BP-PCS, PSQI, and serum BDNF levels (the independent variables). The linear regressions were calculated for EO, EC, and EO-EC conditions. We used a test with 5000 permutations to determine the significance threshold and to correct for multiple comparisons. We used a test with 5000 possible outcomes to find the significance threshold and make up for the fact that we were comparing the same thing more than once. All analyses were performed with two-tailed tests. We accepted a type I error of 5%. We used a two-tailed type I error of 0.05 for all analyses. Furthermore, we used Bonferroni's Multiple Comparison Test to identify the source of significant differences and to adjust for multiple comparisons. For all analyses, we considered a two-sided p-value less than 0.05. The SPSS software version 22.0 (SPSS, Chicago, IL) and the sLORETA software were used to run the analysis.

3. Results

3.1 Spectral power density of the EEG bands according to FM vs. HC groups in EO condition

In the EO condition, the FM group had less activity than the HC group in the alpha-2 band in the cingulate cortex (BA30), and the delta frequency band in the supplementary motor area (BA06). High frequencies of beta-2 and beta-3 show more activity in the supramarginal gyrus (BA40) and the postcentral gyrus (BA02) in people with FM. The data are presented in Table 3 and Fig. 2.

Table 3
The coordinates and Brodmann area of significant source localization of cortical oscillations from the Montreal Neurological Institute (MNI) The data presented the bands of frequency in FM compared to HC in OE, EC, and differences between EO and EC conditions (EO-EC) (n = 64).

							FM	HC	FM-HC	
Condition	**Freq.**	**MNI Coordinates**			**BA**	**Region**	**Mean (SD)**	**Mean (SD)**	**Log-F**	**p**
		x	**y**	**Z**						
EO	delta	65	-5	25	BA06(r)	Supplementary motor area	3.30 (0.36)	3.40 (0.47)	-2.03	< 0.01
	alpha-2	5	-50	15	BA30(r)	Posterior cingulate cortex	0.17 (1.63)	1.37 (3.26)	-2.08	< 0.01
	beta-2	-40	-30	50	BA40(l)	Postcentral gyrus	2.83 (1.63)	0.84 (0.91)	1.96	< 0.001
	beta-3	-40	-30	40	BA02(l)	Postcentral gyrus	2.76 (1.52)	1.39 (0.85)	2.41	< 0.001
EC	delta	10	-60	5	BA30(r)	Superior parietal gyrus	1.18 (3.15	3.85 (3.34)	-2.44	< 0.01
EO-EC	theta	-65	-15	-5	BA21(l)	Middle temporal gyrus	-0.56 (0.20)	-1.20 (0.07)	4.58	< 0.01
	alpha-1	-45	-60	30	BA39(l)	Superior temporal gyrus	-0.43 (0.65)	-1.43 (0.40)	5.44	< 0.01
	alpha-2	35	30	40	BA09(r)	Middle frontal gyrus	-0.29 (0.13)	-0.57 (0.26)	4.60	< 0.01
	beta-3	55	-55	-25	BA37(r)	Fusiform gyrus	-0.14 (0.05)	0.16 (0.19)	-4.23	< 0.01

3.2 Spectral power density of the EEG bands according to FM vs. HC groups in EC condition

In the EC condition, the FM group's delta band (BA30) in the cingulate cortex was much less active than the HC. The data is presented in Fig. 3 and Table 3.

3.3 Spectral power density of the EEG bands according to FM vs. HC groups in the differences between EO and EC conditions (EO-EC)

In the differences between EO and EC conditions, the FM group demonstrates significantly increased brain activity in the theta band placed in the middle temporal gyrus (BA21), as well as over the angular gyrus (BA39) in the alpha-1 band, and in the dorsolateral prefrontal cortex in the alpha-2 band. Over the fusiform gyrus (BA37), the FM group has less activity in the beta-3 band. The data is presented in Table 3 and in Fig. 4.

3.4 Linear and non-linear functional brain connectivity in the EO, EC and differences between EO and EC conditions (EO-EC) according to FM vs. HC subjects

Linear and non-linear functional brain connectivity in the EO, EC and differences between EO and EC conditions according to FM vs. HC subjects is presented in Fig. 5. In the linear analysis in the EO and EC conditions, we did not find any difference in connectivity comparing FM with HC.

In FM, on the beta-3 band, the left insula and right thalamus showed high connectivity in EO-EC condition. The data is presented in Fig. 5A. Similarly, in the differences between EO and EC conditions, the non-linear analysis FM showed improved connectivity in the beta-2 band between the right insula and the left mPFC. The result is presented in Fig. 5B. The non-linear analysis does not show differences between FM and HC groups in EO condition. In the EC condition, FM shows less connection between the right INS and left mPFC in the beta-2 band. The result is presented in Fig. 5C.

3.5 The relationship between linear and non-linear connectivity with the severity of clinical symptoms and BDNF in FM subjects

Linear regression analyses were performed for FM patients between ROI connectivity in distinct frequency bands for mood, pain-related, sleep quality, andserum BDNF. Pain catastrophizing was correlated negatively with linear connectivity between the right ACC and right INS in the beta3 band in the EO condition. Likewise, between right ACC and right S1 in beta 3 band (see, Table 4 and Fig. 4A).

In the EO condition, serum BDNF levels were positively correlated with the non-linear connectivity between the right INS and the left mPFC in the delta band (see Table 4 and Fig. 6B).

In the EC condition, pain disability measured by FIQ has a negative correlated with linear connectivity in the beta-3 band. People with FM who have more pain disabilities have less connection between the left ACC and the left S1 (See Table 4 and Fig. 6C).

In the differences between EO and EC condition, the non-linear connectivity in the beta1 band was negatively linked to depression. FM subjects with elevated BDI scores show decreased activity between left INS and left TH (see Table 4 and Fig. 6D).

Table 4

Linear regression analyses for FM patients between ROI connectivity in different frequency bands for mood, pain-related, sleep quality, and biochemical measures according to EO, EC, and differences between EO and EC conditions (EO-EC) (n = 49). = Unstandardized Coefficients; Cohen r = correlation coefficient: small = 0.1, medium = 0.3, large = 0.5. ACC = anterior cingulate cortex; INS = insula; mPFC = medial prefrontal cortex; S1 = primary somatosensory; TH = thalamus. EO = eyes-open; EC = eyes-closed. *Intercept, and SD values multiplied for (10^4). ** $p < 0.05$.

EO						
CONDITION						
Coherence Connectivity (LLC)						
Dependent Variable: ROI (ACC right ↔ INS right)						
	Intercept	****	**SD**	**Frequency Band**	r	p
Brazilian Portuguese Pain Catastrophizing Scale (BP-PCS)	8.226	-0.043	0.792	Beta3	**-0.513**	**0.006**
Dependent Variable: ROI (ACC right ↔ S1 right)						
	Intercept	****	**SD**	**Frequency Band**	r	p
Brazilian Portuguese Pain Catastrophizing Scale (BP-PCS)	7.263	-0.032	0.578	Beta3	**-0.520**	**0.006**
Phase-Synchronization Connectivity (LNLC)						
Dependent Variable: ROI (INS right ↔ mPFC left)						
	Intercept	****	**SD**	**Frequency Band**	r	p
Brain-derived neurotrophic factor (BDNF)	58.42	1.635	84.30	Delta	**0.533**	**0.040**
EC						
CONDITION						
Coherence Connectivity (LLC)						
Dependent Variable: ROI (ACC left ↔ S1 left)						
	Intercept	****	**SD**	**Frequency Band**	r	p
Fibromyalgia Impact Questionnaire (FIQ)	13.45	-0.068	2.004	Beta3	**-0.493**	**0.027**
EO-EC						
CONDITION						
Phase-Synchronization Connectivity (LNLC)						
Dependent Variable: ROI (INS left ↔ TH left)						

EO						
CONDITION						
Coherence Connectivity (LLC)						
Dependent Variable: ROI (ACC right ↔ INS right)						
	Intercept	β	SD	Frequency Band	r	p
Beck Depression Inventory (BDI)	266	-11.92	219.5	Beta1	**-0.509**	**0.017**

4. Discussion

These findings highlight that in the EO condition, the FM presented decreased spectral power density in delta and alpha-2 bands placed over BA06 and BA30, respectively. In contrast, the beta-2 and beta-3 frequencies in FM have more power spectra than the BA40 and BA02, respectively. In the EC condition, the FM group exhibits decreased spectral power in the delta frequency on the BA30. Besides, in the difference between EO and EC conditions, there is more activity in brain areas that deal with hearing and seeing above BA21, in the beta-1 band, and in the alpha-1 and alpha-2 bands at BA39 and BA09, respectively. In the difference between EO and EC conditions, FM presented higher connectivity in the beta-2 and beta-3 bands between the insula and the mPFC and thalamus, respectively.

Furthermore, FM showed less connection between the insula and the mPFC under EC conditions. These results point out that the insula, mPFC, and thalamus are related to the integration of sensory, emotional, and cognitive components of the FM and that the beta band is involved in the multisensory processing of pain. We also found that the link between the ACC, insula, S1 and thalamus is linked to pain catastrophizing, the effect of FM symptoms on quality of life, depressive symptoms, and serum BDNF levels.

We found decreased delta waves in the premotor and supplementary motor cortex (BA06). These areas contribute to planning complex and coordinated motor movements and the cingulate gyrus (BA30) that composes the retrosplenial cortex (RSC). It connects to the hippocampus, thalamus, and prefrontal cortex [33]. It is an essential part of the brain's core network involved in many functions, such as episodic memory, navigation, creativity, and planning [34]. This measure backs up the fact that people with FM have problems with their working memory, attention, and executive function [35].

Additionally, previous studies using resting-state fMRI and EEG in healthy subjects found higher delta activity over central-parietal areas of the somatomotor network (SMN). At the same time, the alpha and beta bands showed decreases over central-parietal areas extending to frontal regions. Thus, these findings suggest an inverse relationship between low (delta, theta) and high (alpha, beta) EEG frequencies. High rhythms involve cognitive processes such as self-referential attention or memory, while low frequencies involve sensory processing [36]. From this point of view, our results suggest that the FM group is less aroused at the start of the resting state assessment than the HC. Even though we do not have a clear hypothesis so far to accommodate all the differences between FM and HC found across the different

frequency bands, it seems reasonable to derive some conclusions based on the neurophysiopathology of FM. Earlier studies that used other methods, like the EEG spectral density [16], the BOLD signal [13], and the transcranial magnetic stimulation (TMS) parameter [37], support this idea. Ultimately, this set of findings aligns with the notion that cortical hypoactivation may be a marker of cortical dysfunction in FM. It is important to note that they have been linked to the severity of pain disability, the worse symptoms of central sensitization [13], the use of opioids [16], and the severity of the dysfunction of the descending pain modulatory system [37]. Even though the current results add a lot to this field of knowledge, the study design makes it hard to establish cause and effect. On the other hand, these findings are essential for research and clinical settings. They mostly make it possible to use electrophysiological measurements in a controlled experiment, which makes it less likely that assessment biases will affect the results and makes it possible to understand how cortical dysfunction works in nociplastic chronic pain.

The lower activation in the alpha-2 frequency band over areas involved in emotional regulation, sensing, and action (BA30) suggests that precarious emotional regulation of the posterior cingulate cortex (PCC) may come after a prior painful activation of the somatosensory cortex. This finding might explain the catastrophic behavior usually found in FM [38] since the cortical alpha rhythms modulate physiological mechanisms that precede painful motor integration [39]. In contrast to hypoactivation in the cortical areas, the FM group showed increased cross-spectral power in the beta-2 and beta-3 bands over the supramarginal gyrus (BA40) and in the somatosensory cortex (BA02). These areas are involved in preceding visual and tactile stimulation. A previous study found that the higher beta-band power is linked to these regions' functioning to process information from more than one sense [40]. Despite these results' extent of evidence that FM displays a deteriorated function of cortical, indexed by hypoactivation compared to HC, one needs to consider that they may be explained by the sample characteristics related to the severity of disorders, the medication used, disability, comorbidities, etc.

The linear analysis shows that when taking the differences between EO and EC conditions into play, the left insula and right thalamus are more connected in the beta-3 frequency band in the FM group. An fMRI study found that the thalamus is connected to S1, S2, and the insula. It suggests that more activity in the thalamocortical area could cause more activity in the insula, which relates to the long-lasting feeling of pain [41]. It showed that beta- and theta-wave overactivation is linked to thalamocortical dysrhythmia (TCD) in some pain-related areas. For example, when the thalamus function is compromised, these thalamocortical oscillations happen at low theta frequencies and high beta and gamma frequencies. This is how TCD works in pain matrix areas, and it has been suggested that this could lead to chronic pain [42]. These results suggest how people feel, think, and act in response to pain is shaped by how well the different parts of the pain matrix work together. Ong, Stohler, and Herr (2019) [12] found that the pain level during a migraine attack is linked to how well the left insula and the mPFC work together.

On the other hand, in FM, the right insula and left mPFC are more connected in terms of phase synchronization in the beta-2 frequency band. This result is backed up by previous studies showing that the insular cortex integrates sensory information from the corticothalamic ipsi- and contralateral projections. Besides, an earlier study found that the insular cortex combines this information with input from the

prefrontal cortex involved in cognitive, emotional, and executive functions. This integrated work among different brain areas defines voluntary responses to pain [43].

Our research shows changes in how the brain works linked to pain catastrophizing and the effect of FM symptoms on the quality of life (see Table 4 and Fig. 6A). We found that in EO, the beta-3 band is conversely correlated with the linear connectivity between the right ACC, the right insula, and the left S1. According to these results, pain catastrophizing might be related to enhanced functional connectivity (FC) among areas playing a role in pain perception (S1, anterior insula, and thalamus) [44]. They are also linked to an increase in connectivity within the DMN (mPFC-posterior cingulate cortex (PCC)/precuneus) or between the DMN and the periaqueductal gray (PAG)/periventricular gray (PVG) [45]. The DLPFC is one of the most critical parts of the descending pain modulatory system [44]. In the same way, the connectivity between the DMN and the medial thalamus was also related to pain catastrophizing [45]. Neuroimaging studies found that pain catastrophizing leads to changes in the structure and function of the brain. Besides, a cognitive-behavioral therapy study found that pain catastrophizing is associated with more gray matter in the PFC, parietal, and somatosensory cortex [46]. Another study found that chronic pain is related to alterations in gray matter (GM) and FC [47].

In contrast, in the EC condition, connectivity has a negative correlation with the impact of pain on quality of life as measured by FIQ. Our results showed that the connectivity of the beta-3 frequency band in the left hemisphere had an inverse correlation with the connection between ACC and S1 and the impact of fibromyalgia on quality of life (see Table 4 and Fig. 6C). The ACC is involved in several cognitive and emotional processing tasks as a critical part of the limbic system. A previous study found less intrinsic connectivity between the DMN and the insula [10]. Studies found that a higher level of connectivity among cortical areas involved with pain processing is linked to less pain. The ACC has been linked to affective pain because the excitatory activity of its neurons is needed for negative emotions related to pain [48, 49]. However, it has been unclear whether and how the ACC decodes and tells the difference between sensory pain and affective pain.

In the difference between EO and EC conditions, the connection between the left insula and the right thalamus in the beta-1 band was negatively correlated with depression. This finding fits with other evidence suggesting that cortical thickness lateralization may also be linked to depressive symptoms. In a previous study, adolescent girls who later became clinically depressed were found to have thicker gray matter in the left insula than those who did not become depressed [49]. People with depressive symptoms seem to have abnormally lateralized activation in the insula and limbic structures when their emotions are stimulated [50]. In line with these results, a meta-analysis found that the left insula was more active when people were angry, sad, or scared in response to unpleasant stimuli [51]. Previous studies have shown that the insula is involved in processing multidimensional pain aspects, which include sensory, affective, and cognitive dimensions.

In neuroimaging studies of acute experimental pain, the insula is one of the most active parts of the brain [4]. Several studies in EO or EC conditions discovered that low beta band power (13–20 Hz) in chronic neuropathic pain could be a potential brain GABAergic signaling marker [52]. Based on this information, it seems reasonable to think that the severity of FM symptoms like pain, depression, and central sensitization

may be linked to an imbalance in the GABAergic and glutamatergic concentrations in different structures, such as the insula [53]. However, the insula does more than just process pain signals. It has been linked to several unpleasant interoceptive and exteroceptive experiences [54]. So, more research is needed to figure out how left insula thickness and insula asymmetry are related to depressive symptoms and widespread pain.

In the EO condition, the delta band positively correlates with the connectivity between the left insula, the right mPFC, and serum BDNF (see Table 4 and Fig. 6B). This is important because the BDNF protein consistently promotes synaptic plasticity. Even though there is no clear explanation for these results, one needs parsimony to translate them into the clinical setting. According to the literature, delta waves are related to neural plasticity processes. At the same time, people with chronic pain, especially those with nociplastic pain, have higher serum levels of BDNF [55, 56]. Serum BDNF was conversely correlated with the function of the DPMS [57]. Whereas in the spinal cord, the increase of BDNF is allied with a reduction in the GABA inhibitory activity and increased excitability of the spinothalamic tract [58]. In another study with FM, we found that a standard pain stimulus led to more functional connections between the motor areas and the PFC [15]. Roy et al. (2020) [59] found that the right frontal-parietal regions are more likely to have more delta waves in the Met/Met homozygous genotype of BDNF than in the heterozygous genotype.

Several methodological aspects must be addressed in the interpretation of these results: *First*, this study was cross-sectional, so we could not determine whether long-term chronic pain or a more severe disease was responsible for the electrophysiological changes. *Second*, the source localization has a low resolution due to the small number of EEG sensors (18 electrodes). This is enough for source reconstruction, but it makes anatomical accuracy less accurate. *Third*, groups were not matched up and had different years in school or ages. *Fourth*, only women were included because FM is more common in women and because men and women have different ways of dealing with pain, brain activity, and connections [60]. *Fifth*, it is not possible to control all possible confounding factors. Antidepressants, painkillers, mood stabilizers, and antipsychotic medications are a few of these factors that affect people with FM. Lastly, because this is a cross-sectional study, more longitudinal research is needed to figure out the role of pain matrix connectivity as a predictor of how chronic pain will change over time.

These results mainly indicate the insula's role in the connectivity integration of different pain processing circuits in FM and point out the beta band activity taking part in this process. So, these results suggest that the insula functional connectivity at rest could be an objective biomarker of how pain is processed and related to the severity of clinical symptoms in people with FM.

Declarations

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

R.L.A, M.Z. and W.C. conceived and designed the study, participated in the sequence alignment, performed the statistical analysis, and coordinated and drafted the manuscript; R.L.A. P.V.S. and M.Z. collected and registered the data; R.B.T. and R.P.A helped in the data preprocessing; I.L.S.T. and F.F. contributed to study conception and design, the interpretation of results, and reviewed the manuscript; R.L.A., M.Z. and W.C. made the final review.

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ADDITIONAL INFORMATION

The authors declare no competing interests.

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Figures

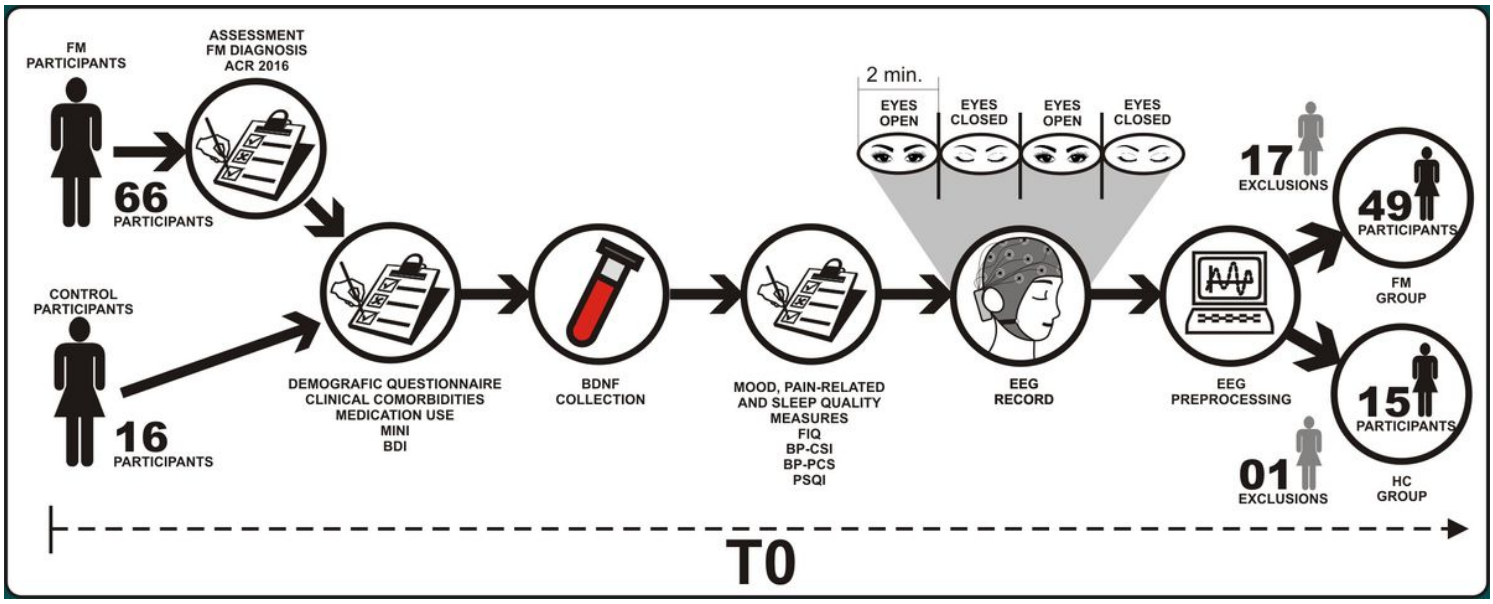

Figure 1

Flowchart of the study assessments. FM = Fibromyalgia; HC= Health Controls; ACR = American College of Rheumatology; FIQ = Fibromyalgia Impact Questionnaire; BDI = Beck Depression Inventory; MINI = Mini International Neuropsychiatric Interview; BP-PCS = Pain Catastrophizing Scale; BP-CSI = Central sensitization inventory—Brazilian Portuguese version; PSQI = Pittsburgh Sleep Quality Index; BDNF = brain-derived neurotrophic factor.

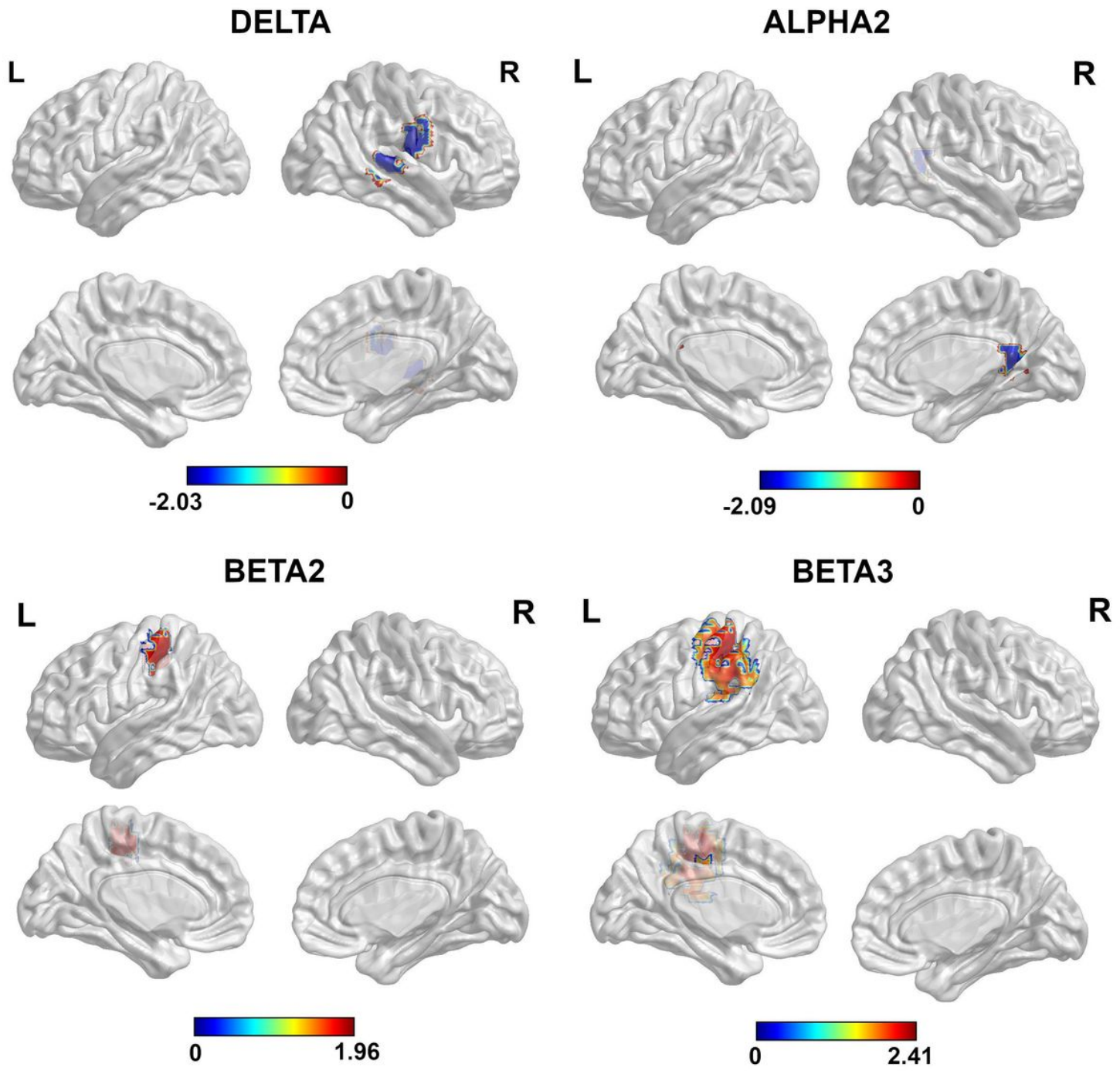

Figure 2

Statistical maps of FM group cortical oscillations for eyes open (EO) to the delta, alpha2, beta2, and beta3 frequency bands. MRI slices were placed at the MNI-space coordinates. The colored area represents the voxel with the highest significance. Values are given in log F-ratio values (threshold FM>HC: log-F = 1.73, $p<0.001$; FM<HC: log-F= -1.69, $p<0.01$). L, left; R, right. ACC = anterior cingulate cortex; INS = insula; mPFC = medial prefrontal cortex; S1 = primary somatosensory; TH = thalamus.

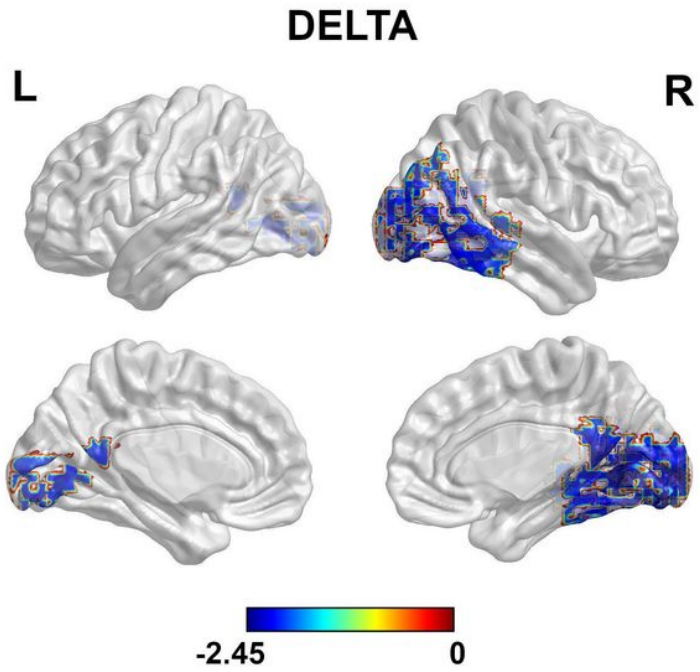

Figure 3

Statistical maps of cortical oscillations of the FM group for eye's closed condition (EC) to the delta frequency band. The colored area represents the voxel with the highest significance. Values are given in log F-ratio values (threshold FM<HC: log-F= 1.95, p<0.005). L, left; R, right. ACC = anterior cingulate cortex; INS = insula; mPFC = medial prefrontal cortex; S1 = primary somatosensory; TH = thalamus.

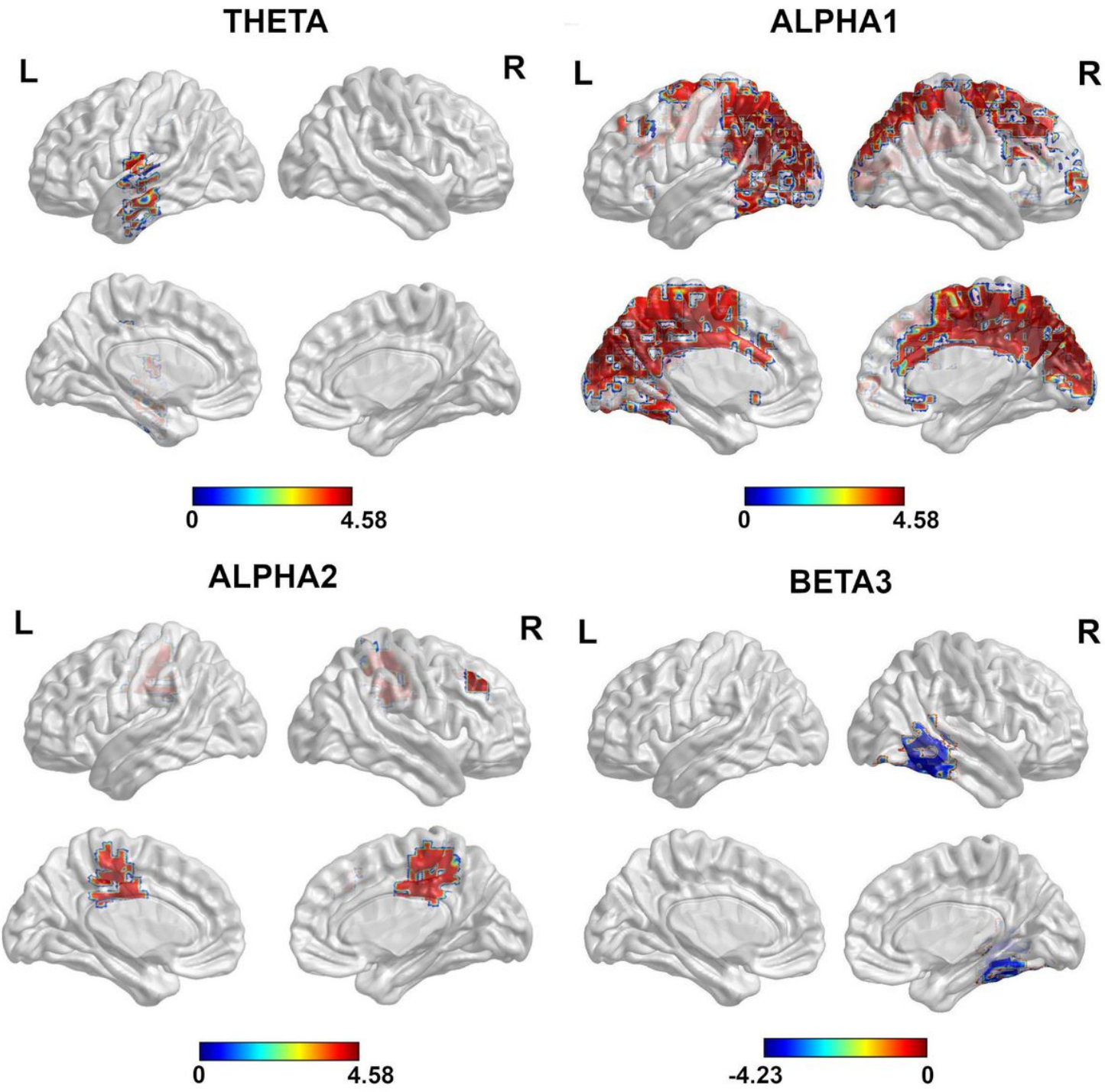

Figure 4

Statistical maps of cortical oscillations of the FM for the EO minus EC condition (EO-EC) to theta, alpha-1, alpha-2, and beta-3 frequencies. The colored area represents the voxel with the highest significance. Values given in log F-ratio values (threshold FM>HC: log-F = 3.86, p<0.001; FM<HC: log-F = -3.44, p<0.01). L, left; R, right. ACC = anterior cingulate cortex; INS = insula; mPFC = medial prefrontal cortex; S1 = primary somatosensory; TH = thalamus.

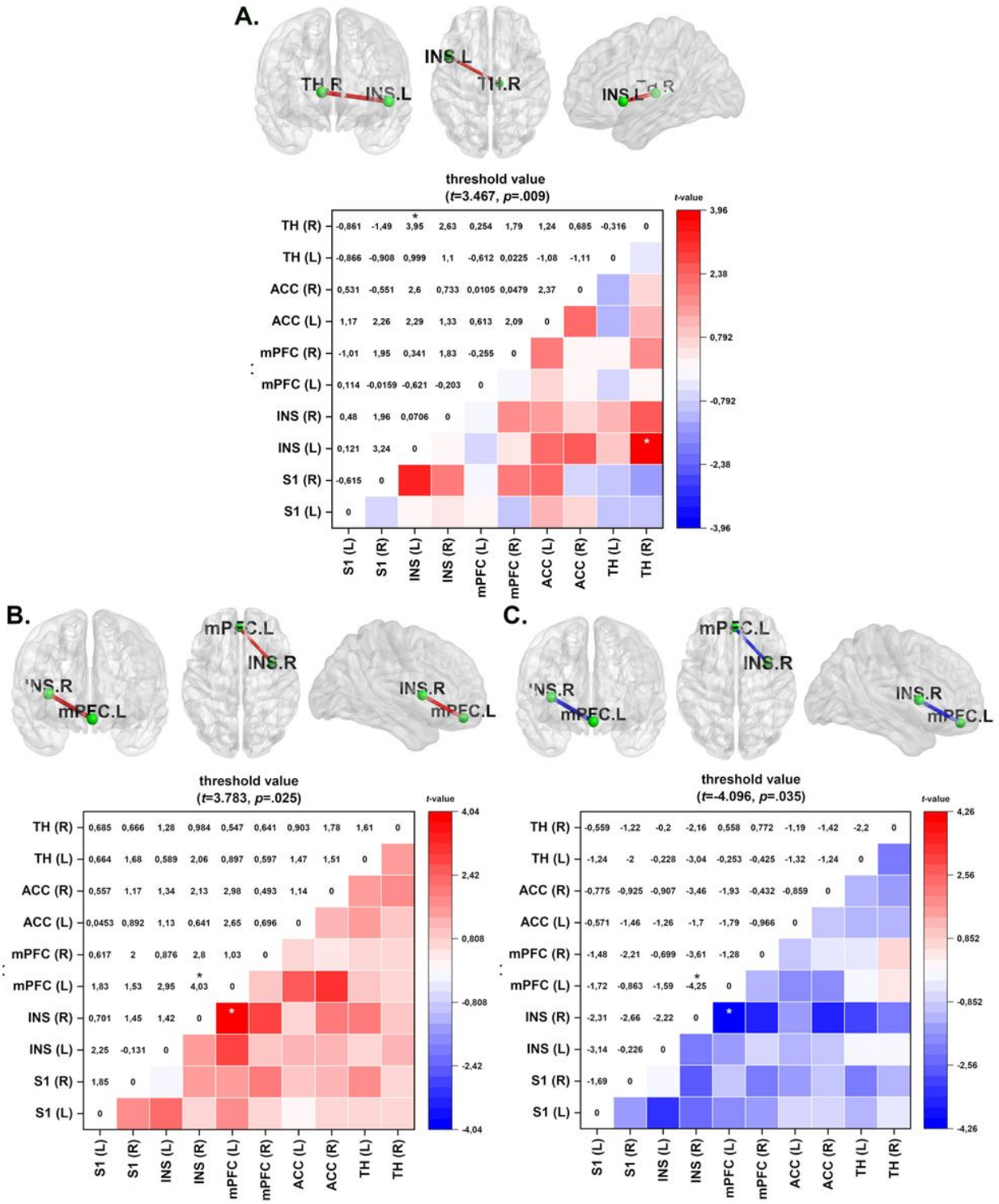

Figure 5

Connectivity maps and color maps of the FM group compared with HC. **A.** Linear analysis in EO-EC condition shows enhanced connectivity between left INS and right TH in the FM group in the beta3 frequency band. The colored edge represents connections with significant differences. **B.** Non-linear analysis in the EO-EC condition shows increased connectivity for the FM group among the right INS and the left mPFC in the beta2 band. **C.** Non-linear analysis in EC condition, FM group exhibits decreased connectivity in the beta2 band of

the right insula and left mPFC. Values are given in t-values. ACC = anterior cingulate cortex; INS = insula; mPFC = medial prefrontal cortex; S1= primary somatosensory; TH = thalamus. *p<0.05.

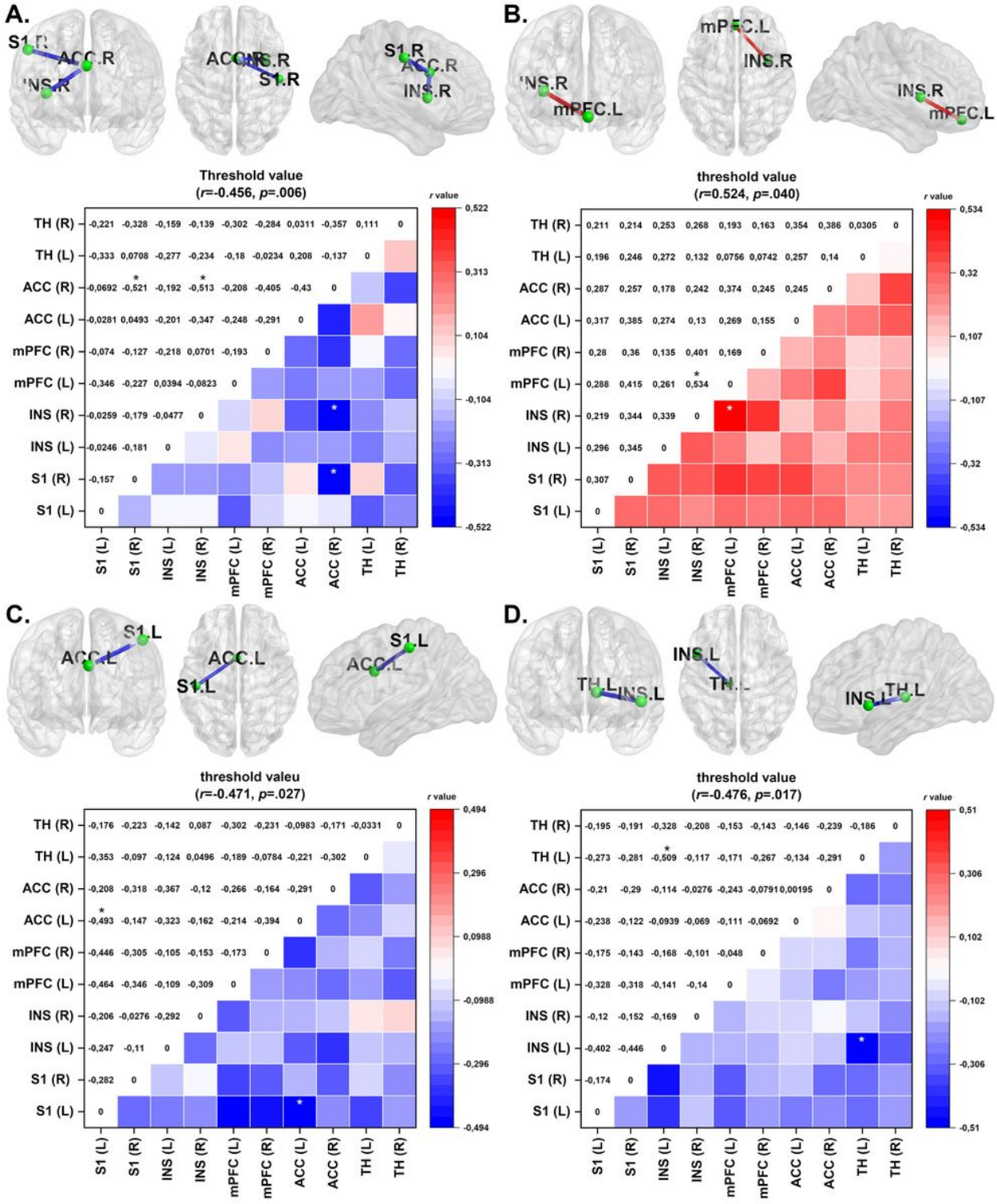

Figure 6

Connectivity and color maps of the linear regression analyses for FM patients between ROI connectivity and mood, pain-related symptoms, sleep quality, and biochemical measures. In EO condition: **A.** Diminished coherence connectivity in the beta-3 band between the right ACC, right INS and right S1 negatively correlates

with BP-PCS. **B.**An increased phase-synchronization connection among left INS and left TH in the delta band correlates with serum BDNF levels in EO condition. **C.** Decreased coherence connections in the beta-3 band between the left ACC and left S1 negatively correlate with FIQ in EC conditions. **D.** The lower phase-synchronization connection in the beta1 band correlates inversely with BDI in EO-EC condition. Values given in Cohen r = correlation coefficient (small=0.1; medium=0.3; large=0.5). ACC = anterior cingulate cortex; INS = insula; mPFC = medial prefrontal cortex; S1 = primary somatosensory; TH = thalamus. *p<0.05.