

RESEARCH SUBMISSION

Early effect of onabotulinumtoxinA on EEG-based functional connectivity in patients with chronic migraine: A pilot study

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Abstract

Objective: In this pilot prospective cohort study, we aimed to evaluate, using high-density electroencephalography (HD-EEG), the longitudinal changes in functional connectivity (FC) in patients with chronic migraine (CM) treated with onabotulinumtoxinA (OBTA).

Background: OBTA is a treatment for CM. Several studies have shown the modulatory action of OBTA on the central nervous system; however, research on migraine is limited.

Methods: This study was conducted at the Neurology Unit of "Policlinico Tor Vergata," Rome, Italy, and included 12 adult patients with CM treated with OBTA and 15 healthy controls (HC). Patients underwent clinical scales at enrollment (T0) and 3 months (T1) from the start of treatment. HD-EEG was recorded using a 64-channel system in patients with CM at T0 and T1. A source reconstruction method was used to identify brain activity. FC in δ - θ - α - β -low- γ bands was analyzed using the weighted phase-lag index. FC changes between HCs and CM at T0 and T1 were assessed using cross-validation methods to estimate the results' reliability.

Results: Compared to HCs at T0, patients with CM showed hyperconnected networks in δ ($p=0.046$, area under the receiver operating characteristic curve [AUC: 0.76-0.98], Cohen's κ [0.65-0.93]) and β ($p=0.031$, AUC [0.68-0.95], Cohen's κ [0.51-0.84]), mainly involving orbitofrontal, occipital, temporal pole and orbitofrontal, superior temporal, occipital, cingulate areas, and hypoconnected networks in α band ($p=0.029$, AUC [0.80-0.99], Cohen's κ [0.42-0.77]), predominantly involving cingulate, temporal pole, and precuneus. Patients with CM at T1, compared to T0, showed

Abbreviations: BDI, Beck Depression Inventory; BS, bootstrap; BTA, botulinumtoxin-A; CM, chronic migraine; CV, cross-validation; EC, eyes closed; FC, functional connectivity; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; FWE, family-wise error; HADS, Hospital Anxiety and Depression Scale; HC, healthy control; HD-EEG, high density electroencephalography; HIT-6, Headache Impact Test; MIDAS, Migraine Disability Assessing Scale; MMDs, monthly migraine days; mNC, mean network connectivity; NBS, network-based statistics; OBTA, onabotulinumtoxinA; PREEMPT, Phase III Research Evaluating Migraine Prophylaxis Therapy; ROC, receiver operating characteristic; ROI, region of interest; RS, resting state; VAS, Visual Analogue Scale.

Matteo Conti and Roberta Bovenzi contributed equally as co-first authors.

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hypoconnected networks in δ band ($p=0.032$, AUC [0.73-0.99], Cohen's κ [0.53-0.90]) and hyperconnected networks in α band ($p=0.048$, AUC [0.58-0.93], Cohen's κ [0.37-0.78]), involving the sensorimotor, orbitofrontal, cingulate, and temporal cortex. **Conclusion:** These preliminary results showed that patients with CM presented disrupted EEG-FC compared to controls restored by a single session of OBTA treatment, suggesting a primary central modulatory action of OBTA.

Plain Language Summary

Chronic migraine, a disabling neurological condition, can be treated with onabotulinumtoxin A (OBTA) which works by blocking the release of pain-related substances from a major nerve of the head. This study aimed to learn how OBTA affects the message pathways in the brain by comparing the electrical activity in the brains of patients with migraine who were treated with OBTA and the electrical activity in the brains of healthy people. We found that patients with migraine had significant changes in several brain areas that were restored after just one session of OBTA treatment, confirming a primary action of OBTA on the brain's electrical activity.

KEYWORDS

central effects, chronic migraine, high-density electroencephalography, onabotulinumtoxinA

INTRODUCTION

Chronic migraine (CM) is a highly debilitating neurological condition characterized by persistent headaches occurring on at least 15 days each month, with migraine-like features for at least 8 days, for more than 3 months.¹⁻³ CM is marked by a disruption of both peripheral and central nociceptive networks.² Indeed, the repetitive release of various neuropeptides by the initial trigeminal nociceptive neurons during migraine attacks, described as "peripheral sensitization,"⁴ leads to maladaptive changes in the central trigeminal pathway, defined as "central sensitization," which in turn leads to an abnormal response to subthreshold sensory inputs.⁵

The administration of onabotulinumtoxinA (OBTA), following the Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) protocol, is a safe and effective prophylactic treatment for CM.⁶ While randomized placebo-controlled studies have demonstrated the clinical efficacy of OBTA, our understanding of the mechanisms underlying its therapeutic role in CM remains incomplete. By blocking the repeated release of pro-inflammatory and vasoactive neuropeptides in the peripheral pain pathways, OBTA leads to a reduction in the phenomenon of peripheral sensitization;^{2,7} however, whether the suppression of peripheral sensitization also has a secondary effect on inhibiting the development and maintenance of central sensitization is still a matter of debate.⁸

Recent studies using novel approaches to analysis demonstrated that a network-based estimation of functional connectivity (FC) can be reliably performed in physiological and pathological conditions in humans,⁹ with the potential to enhance our comprehension of the

underlying mechanisms of the disease.^{10,11} Furthermore, assessing FC abnormalities in patients with CM and their possible modifications with OBTA treatment would provide further insights into the central mechanism of action of OBTA. Indeed, in the case of dystonia and spasticity, numerous neuroimaging and neurophysiological studies have investigated the central effects of OBTA.^{12,13} In contrast, when it comes to CM, studies investigating the central effects of OBTA are notably scarce and mainly limited to a few neurophysiological studies.^{14,15}

The study of FC is primarily based on radiological techniques such as functional magnetic resonance imaging (fMRI) or electrophysiological techniques such as high-density electroencephalography (HD-EEG). fMRI is based on indirect measures of neural activity, and its temporal resolution is suboptimal to capture brain dynamics, which occur in a few milliseconds. HD-EEG, a non-invasive and accessible method to evaluate cortical electrical activity, overcomes the limitations, as it can detect subtle dysfunctions in cortical processing with high temporal resolution.^{16,17}

We hypothesized that the OBTA treatment could induce functional modification in patients with CM. The primary aim of this pilot study was to provide novel insights into the central mechanisms of action of OBTA in CM treatment. To this purpose, we used advanced neurophysiological techniques (HD-EEG) to provide a detailed characterization of resting-state FC abnormalities in a cohort of adult patients with CM and to investigate the FC longitudinal changes following OBTA treatment. Despite the study's current restriction primarily due to the small number of patients included, our goal was to advance our understanding of CM pathogenesis and shed light on the therapeutic implications of OBTA in modifying brain connection patterns.

METHODS

Study subjects

This prospective cohort study was conducted at the Neurology Unit of "Policlinico Tor Vergata," Rome, Italy, between January 2023 and November 2023. It was conducted a priori as a primary analysis of the data collected, and no previous manuscript has reported any aspects of the data used in this study.

Inclusion criteria were: (1) patients of both sexes of legal age (>18 years); (2) diagnosed with CM in accordance with the criteria of the International Headache Society (International Classification of Headache Disorders, 3rd edition)¹; (3) initiating treatment with OBTA according to clinical practice and following the therapeutic indications currently authorized by the Italian Medicines Agency (AIFA); (4) no migraine attack or acute medication intake 72 h before the start of the protocol, to exclude the post-ictal phase. Exclusion criteria were: (1) known hypersensitivity to OBTA; (2) history of other neurological or psychiatric conditions; (3) altered consciousness, organ failure, or severe infectious diseases that could preclude the clinical pathway; (4) treatment with migraine preventive drugs in the previous 3 months; (5) medication overuse¹; (6) migraine attack within 48 h after the EEG recording, to exclude the pre-ictal phase.

As a control group, we enrolled age- and sex-matched healthy subjects, without any history of neurological, psychiatric, internal conditions, or cancer. These subjects were recruited specifically for this purpose, and their data had not been previously published. Moreover, the groups were designed to be similar based on specific criteria such as demographic characteristics and clinical profiles.

Fifty-six subjects (30 patients with CM and 26 healthy controls [HCs]) were considered eligible for the study. Twelve adult patients diagnosed with CM and initiating OBTA treatment according to clinical practice and 15 adult HCs were finally included in this study. The recruitment process is summarized in the flow diagram of Figure 1.

The study was conducted in agreement with the principles of the Declaration of Helsinki. The ethical committee of Policlinico Tor Vergata approved the study (protocol code RS 16/17). All participants signed the informed consent.

OBTA treatment protocol

Patients with CM received a minimum intramuscular dose of 155 IU of OBTA administered to 31 injection sites across seven head and neck muscles using a fixed-site and fixed-dose injection paradigm (each injection was 5 IU in 0.1 mL saline) in accordance with the PREEMPT injection paradigm.⁶

Assessments

All patients underwent a detailed medical history collection related to the clinical characteristics of migraine episodes (duration,

days of migraine per month, number of symptomatic drugs taken, location and quality of pain, presence of accompanying symptoms, autonomic and/or dopaminergic symptoms, presence of cutaneous allodynia), previous preventive treatments, comorbidities, and concomitant therapies. A routine neurological examination was conducted to exclude the presence of focal neurological deficits, and an assessment of disability related to migraine was carried out using the following standardized scales validated in accordance with common clinical practice: the Migraine Disability Assessing Scale (MIDAS)¹⁸ and the Headache Impact Test (HIT-6)¹⁹ for quantifying the impact of migraine on daily life activities; the Beck Depression Inventory (BDI) scale and the Hospital Anxiety and Depression Scale (HADS)²⁰ for the identification and quantification of any associated anxious and depressive symptoms; the Visual Analogue Scale (VAS) for the evaluation of pain intensity; the monthly migraine days (MMDs) for the migraine frequency.

Each patient was asked to maintain a daily diary from 3 months before treatment (T0) until the end of the study (T1) to analyze the following parameters: days of migraine, intensity and duration of the attack, number of symptomatic drugs taken, presence of associated symptoms, and any triggering factors. The diaries were written in pen.

There were no missing data from the diaries or the clinical assessments.

Definition of responders

In line with previously accepted criteria,^{21,22} we identified two groups of patients: "responders" ($n=5$), defined as those patients who achieved a $\geq 50\%$ reduction in MMDs during the 3 months after the OBTA treatment compared to the 3 months before the initiation of treatment, and "non-responders" ($n=7$), defined as those patients who did not achieve a $\geq 50\%$ reduction in MMDs.

EEG acquisition protocol

HD-EEG data were recorded in patients with CM for 10 min at a sampling rate of 1024 Hz, band-pass filtered at 0.5–50 Hz using a 64-channel EEG system (EbNeuro BePlus-ProStandard). Electrodes were positioned according to the 10–10 International System.²³ Impedance was kept below 5 k Ω . In line with previous EEG studies,^{9,24,25} HD-EEG recording was performed in the eyes closed (EC) resting state (RS): the subjects were instructed to keep their eyes closed while staying awake for 5 min. Then, reactivity to eyes opening and activation tests were performed to exclude the appearance of epileptiform elements in the EEG data. EEG were recorded in patients with CM in the interictal phase (at least 72 h after and 48 h before a migraine attack or acute medication intake). Patients were contacted by phone 48 h after the EEG recording to ensure that they had not had a migraine attack. Indeed, patients with CM in a pre-ictal phase were excluded from

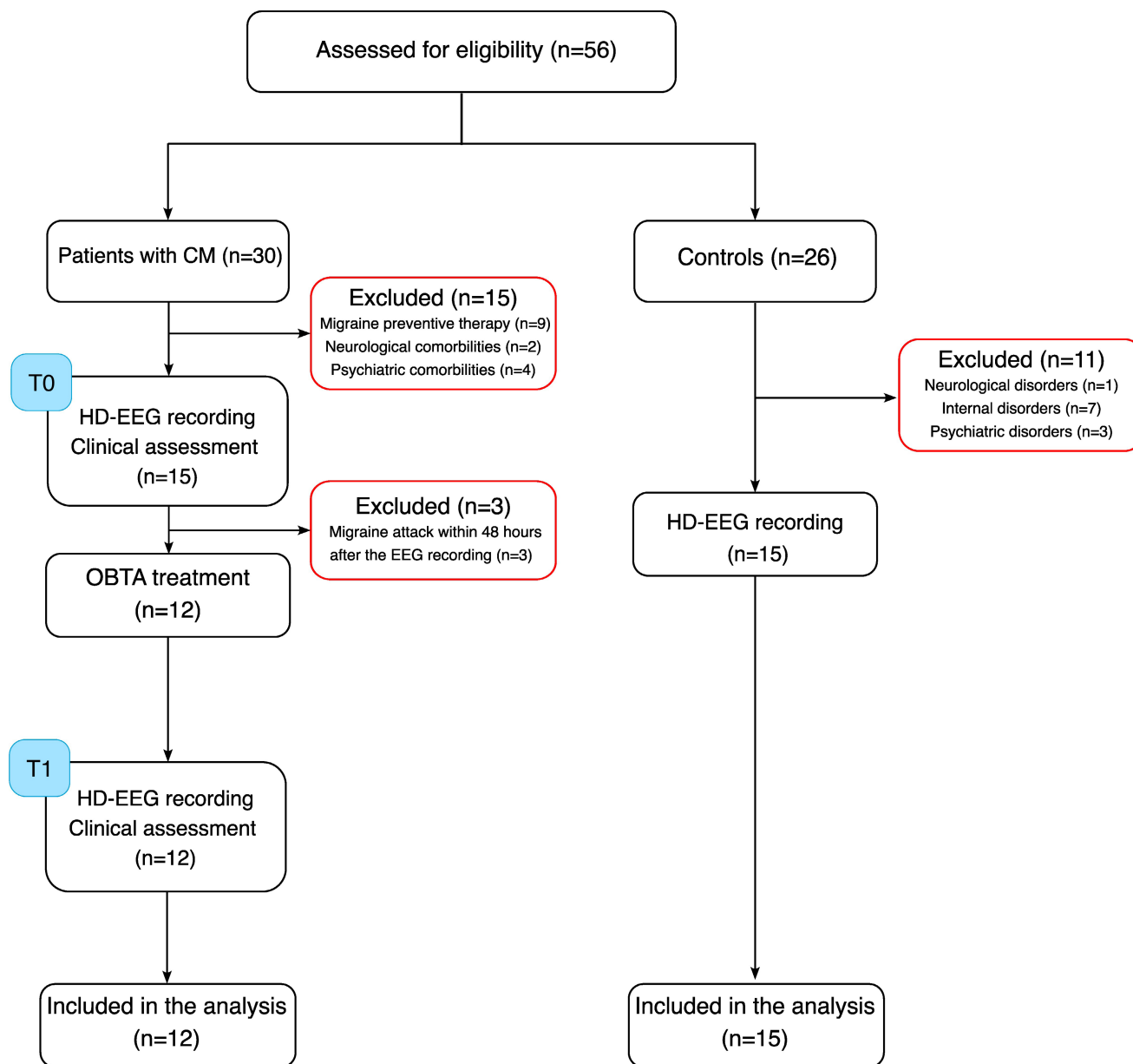


FIGURE 1 Flow chart of the recruitment process of the study. CM, chronic migraine; HD-EEG, high-density electroencephalography; OBTA, onabotulinumtoxinA. [Color figure can be viewed at wileyonlinelibrary.com]

the analysis, as indicated in Figure 1. The same HD-EEG recording was obtained in HC.

Both the HD-EEG recordings and the clinical evaluations were performed at the baseline screening visit (enrolment–T0), just before the start of OBTA treatment, and at 3 months (T1) from the start of OBTA therapy (Figure 2).

FC analysis

We then proceeded to the primary FC analysis of the data collected. The data analyses were performed a priori and blind to the clinical examinations.

HD-EEG recordings in the EC condition were segmented into epochs of 30s each for visualization purposes.²⁴ The first epoch of each recording was discarded. Then, we manually selected the first six consecutive low-artifact epochs (total 180s), that were retained for the following analysis. The same method was used for patients with CM and HCs.

Independent component analysis was used to remove the residual EEG artifacts.²⁶ Then, we proceeded with EEG source localization.¹⁷

EEG channels and MRI template (ICBM152) were co-registered through the identification of the same anatomical landmarks, and the boundary element method²⁷ was used to solve the forward problem. We used weighted minimum-norm estimation to solve the

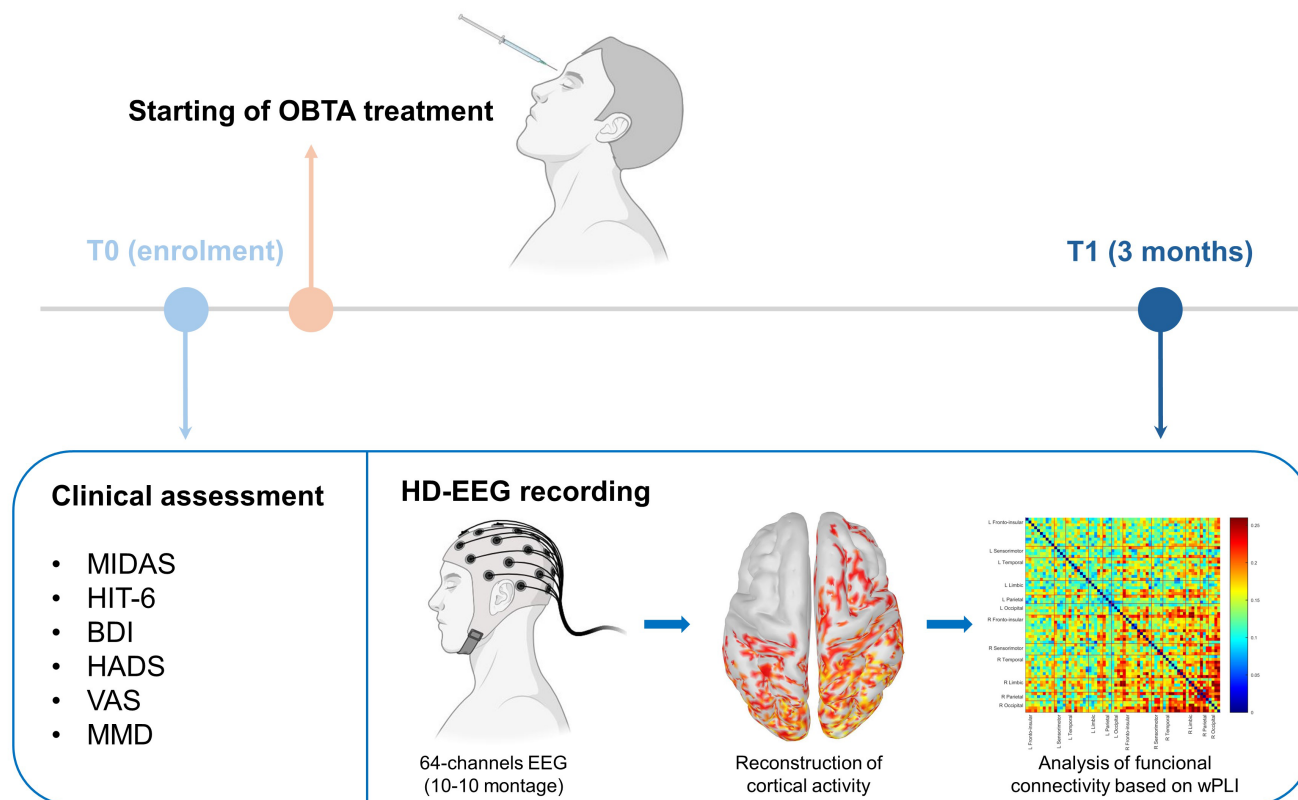


FIGURE 2 Pipeline of the study protocol. In each patient with CM, the HD-EEG recordings and the clinical evaluations were performed at the baseline screening visit (enrolment–T0), just before the start of OBTA treatment, and at 3 months (T1), from the start of OBTA therapy. Cortical activity was reconstructed from the EEG signal. Then, FC matrices for each band was computed using weighted phase lag index (created with BioRender.com). BDI, Beck Depression Inventory; CM, chronic migraine; FC, functional connectivity; HADS, Hospital Anxiety and Depression Scale; HD-EEG, high-density electroencephalography; HIT-6, Headache Impact Test; MIDAS, Migraine Disability Assessment Test; OBTA, onabotulinumtoxinA; VAS, Visual Analogue Scale; wPLI, weighted phase lag index. [Color figure can be viewed at wileyonlinelibrary.com]

inverse problem.²⁸ The sources obtained were divided into 68 brain regions, using the Desikan–Killiany atlas.²⁹

FC was calculated in source space using the values of weighted phase lag index, a measure known to reduce conduction volume artifacts, noise artifacts, and bias from small samples.³⁰ Phase information from the preprocessed signals had been computed using the Hilbert transform in δ (0.5–4 Hz), θ (4–8 Hz), α (8–13 Hz), β (13–30 Hz), and low- γ (30–45 Hz) frequency bands. Dynamic FC matrices were computed between any pair of regions in the above EEG bands, on segments of 1-s length, with an overlap of 50%, according to Welch's method. Of note, this method is not affected by the length of the displayed epochs.³¹ Those matrices were averaged across time epochs to obtain static FC matrices.

Statistical analysis

First, we performed a descriptive analysis, analyzing the mean, median, standard deviation, and percentiles of the study variables. Because the Kolmogorov–Smirnov test demonstrated a non-normal distribution of the variables, we used a nonparametric statistic. Differences in sex and age between HCs and CM groups were tested

using the chi-square and the Mann–Whitney U tests, respectively. Differences in clinical scales (MIDAS, HIT-6, BDI, HADS, VAS) and MMD of CM at T0 and T1 time points were examined using the Wilcoxon test.

Differences in δ , θ , α , β , and low- γ FC between patients with CM and HCs were analyzed using the network-based statistic (NBS). The standard mass-univariate analysis involves the order of thousands of multiple comparisons, with a high risk of family-wise error (FWE). For this reason, we employed NBS to control FWE. NBS is a cluster-based statistical method^{24,32,33} that has been shown to provide greater statistical power than traditional correction methods, such as Bonferroni or false discovery rate (FDR).³⁴ In this study, we used 2.1 as the initial threshold τ and 5000 permutations for the permutation test. For each group comparison at different frequencies, we considered multiple networks from the initial threshold ($\tau=2.1$) to the highest possible threshold (i.e., threshold that identified an existing network with at least links >1). Then, we selected the NBS network with the lowest τ value and $p < 0.05$ to identify the largest differential network with statistical significance. This experimental design was in accordance with previous NBS-based studies.^{24,35} Age and sex were considered confounding factors in NBS.

Moreover, we defined the mean network connectivity (mNC) as the average connectivity of an NBS network. We analyzed the Spearman correlation between mNC of the altered network of CM compared to controls at T0 and clinical features of CM, in particular MIDAS, HIT-6, VAS, HADS, BDI, and MMD. Then, we analyzed the Spearman correlation between the differences at T1 and T0 of mNC of the differential network between CM-T1 and CM-T0 (Δ mNC) and the differences at T1 and T0 of clinical features (Δ MIDAS, Δ HIT-6, Δ VAS, Δ HADS, Δ BDI, and Δ MMD). Finally, we analyzed the differences of Δ mNC between the responders and the non-responders using the Mann-Whitney *U* test. The FDR method was used to correct for multiple comparisons and control the increase of type I error.

All statistical analyses were conducted using two-tailed tests, and a significance level of $p < 0.05$ was employed throughout the study. No statistical power calculation was conducted prior to the study. The sample size was based on the available data and on our previous experience with the design of an FC study based on NBS.

Cross-validation methods

We evaluated the reliability of our NBS results through a novel tool called NBS-Predict.³⁶ NBS-Predict employs suprathreshold edge selection in the training set in the outer loop to identify a connected component among the set of suprathreshold edges. The suprathreshold edge selection is identical to the original NBS method (more details on this method can be found in Serin et al.³⁶). The model for predictive analysis was linear discriminant analysis. The cross-validation (CV) algorithm we used in our study is the leave-one-out, an exhaustive CV method. Using NBS-Predict, we estimated the following metrics for each network CV to differentiate one group versus

another: accuracy (proportion of correctly identified links relative to the total number of links),³⁷ precision (proportion of true positive links relative to all links identified as positive),³⁸ Cohen's kappa (a statistic metric that measures the agreement between observed and expected classifications, correcting for the possibility of agreement occurring randomly),³⁹ sensitivity (proportion of true positive links correctly identified), specificity (proportion of true negative links correctly identified), and receiver operating characteristic (ROC) curve (graphical representation illustrating the trade-off between sensitivity and specificity across various threshold settings).

Moreover, we estimated the out-of-sample performance of the Spearman correlation analysis bootstrapping (BS) as a CV method. This resampling technique allowed us to assess the stability and generalizability of our correlation estimates by repeatedly sampling subsets of the data and simulating different training and testing datasets. The number of BS iterations we used was 1000. In the results, we provided a 95% confidence interval (BS-95%CI) of the BS distribution of correlation coefficients, as an estimation of the robustness of our findings.

The analysis was performed using a custom-written script based on MATLAB 2022a, combined with Brainstorm, NBS, and NBS-Predict toolboxes.^{34,36,40} Graphs were based on MATLAB 2022a and R (ggplot2 package). Details on the methods of the present study can be found in this previous study by our group.⁹

RESULTS

Table 1 reports the demographic and clinical characteristics of HCs and patients with CM, including the clinical scales of patients with CM at baseline (CM-T0) and at 3 months after OBTA treatment (CM-T1).

TABLE 1 Clinical and demographic data of the study population.

	CM-T0 (n = 12)	CM-T1 (n = 12)	HCs (n = 15)	p-value
Age	40.5 [29.8 48.8]	/	42.0 [30.3 49.5]	0.542
F/M	8/4	/	10/5	0.681
Age at onset	12.5 [10.0 16.0]	/	/	/
Allodynia (yes/no)	8/4	/	/	/
Responders/non-responders	/	5/7	/	/
MMD	17.5 [15.0 23.0]	8.5 [6.0 10.8]	/	0.005*
VAS	8.0 [7.0 8.5]	7.5 [6.0 8.0]	/	0.119
MIDAS	51.0 [20.0 69.0]	13.5 [9.3 18.8]	/	0.004*
HIT-6	65.0 [57.0 72.5]	59.0 [53.8 63.0]	/	0.029*
BDI	14.0 [4.8 21.3]	8.0 [3.0 19.5]	/	0.036*
HADS	19.0 [15.8 23.0]	14.5 [11.5 18.8]	/	0.138

Note: Data are expressed as median [25th, 75th percentiles] of variables. Age is expressed in years.

Abbreviations: BDI, Beck Depression Inventory; CM-T0, chronic migraine at baseline; CM-T1, chronic migraine 3 months after treatment; F, females; HADS, Hospital Anxiety and Depression Scale; HCs, healthy controls; HIT-6, Headache Impact Test; M, males; MIDAS, Migraine Disability Assessment Test; MMD, monthly migraine days; VAS, Visual Analogue Scale.

*Statistical significance.

Comparison between HCs and patients with CM

FC at δ , α , β bands was significantly different between patients with CM at baseline (CM-T0) and HCs. Through NBS analysis, we identified a statistically significant network at the δ band (2.1, $p=0.046$), where the connectivity was higher in CM-T0 than in controls. The network was composed of 63 nodes and 170 edges and slightly lateralized in the right hemisphere (51%). Regions of interest (ROIs) with the highest number of connections (degree) were mainly part frontal (in particular right orbitofrontal cortex), sensorimotor (mainly right pre- and postcentral gyrus), occipital (bilateral cuneus and pericalcarine gyrus), and temporal (prevalent bilateral temporal poles) lobes. Intra-frontal (13%) and fronto-occipital (11%) were the predominant connections of this network (Figure 3A,B).

In the α band, we found a significant network (τ 2.2, $p=0.029$), characterized by lower FC in CM than controls. This network included 59 nodes and 135 links, and slight lateralization in the left hemisphere (53%). Regions with higher degrees were located in limbic (predominantly bilateral posterior areas and isthmus of cingulate gyrus), temporal (prevalently bilateral temporal pole and superior

temporal sulcus), and parietal (mostly right precuneus) lobes, with dominant temporo-parietal (9%), temporo-limbic (8%) and intra-temporal (7%) links (Figure 3C,D).

Finally, we identified a significant network in the β band (τ 2.4, $p=0.031$), where FC was higher in CM than in controls. The network was composed of 51 nodes and 91 links and was slightly lateralized in the right hemisphere (51%). The highest degree ROIs of this network were part of frontal (in particular left frontal pole and bilateral orbitofrontal cortex), sensorimotor (mainly left pre- and postcentral gyrus), occipital (in particular bilateral superior frontal areas), temporal (mostly right transverse and superior temporal gyrus), and limbic (mainly bilateral anterior cingulate), with prevalent connections in fronto-occipital (16%) and temporo-occipital (10%) links (Figure 3E,F).

A significant positive correlation between mNC of the δ FC network (CM-T0>HC, τ 2.1) and MIDAS score at T0 was found ($r=0.60$, $p=0.037$, BS-95%CI [-0.032 0.87]). Moreover, we observed a significant positive correlation between mNC of the β FC network (CM-T0>HC, τ 2.4) and VAS score at T0 ($r=0.56$, $p=0.049$, BS-95%CI [0.11 0.72]). No other correlations between mNC of altered network and clinical scores were found.

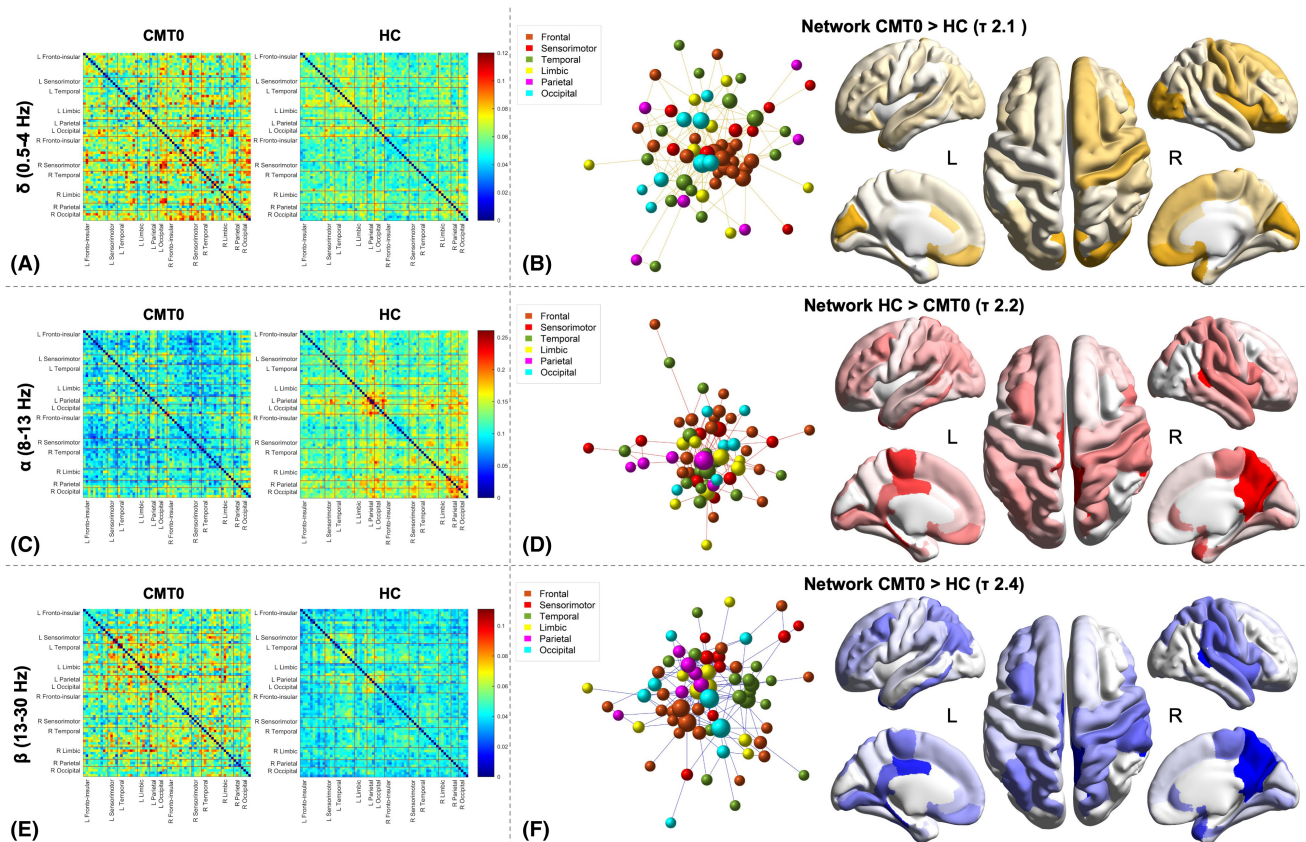


FIGURE 3 Mean FC matrices of CM-T0 and HC groups in δ (A), α (C) and β (E) bands. Differential network between CM-T0 and HC groups in δ (CM-T0>HC) (B), α (HC>CM-T0) (D) and β (CM-T0>HC) (F) bands. The left part of each panel shows graph representation of the differential networks. Colors denote different brain lobes: orange=fronto-insular, red=sensorimotor, yellow=limbic, green=temporal, magenta=parietal, light-blue=occipital; sphere diameters are directly proportional to region of interest (ROI) degrees. The right part of each panel represents ROIs degree of differential networks on model brain surface. CM-T0, chronic migraine at baseline; CM-T1, chronic migraine 3 months after treatment; FC, functional connectivity; HC, healthy control. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

No differences in FC emerged between HCs and patients with CM at T1.

Comparison between T0 and T1 in patients with CM

Compared to T0, at T1, patients with CM showed lower FC in the δ band in a network (τ 2.1, $p=0.032$) composed of 62 nodes and 166 links. The network was lateralized in the right hemisphere (53%). ROIs with the highest degrees were mainly part of frontal (bilateral orbitofrontal and pars orbitalis cortex), sensorimotor (in particular

right postcentral gyrus), limbic (bilateral posterior cingulate), and temporal (in particular right temporal pole) lobes. Intrafrontal (15%), fronto-sensorimotor (10%), and sensorimotor-limbic (7%) were the predominant connections of this network (Figure 4A,B). Patient with CM mNCs of the δ differential networks between CM-T1 and CM-T0 are shown in Figure 4E.

Moreover, we found a significant network in the α band (τ 2.1, $p=0.048$), where FC was higher in CM at T1 compared to T0. This network included 55 nodes and 126 edges and was lateralized in the right hemisphere (56%). The highest degree ROIs of this network were part of frontal (in particular bilateral orbitofrontal cortex

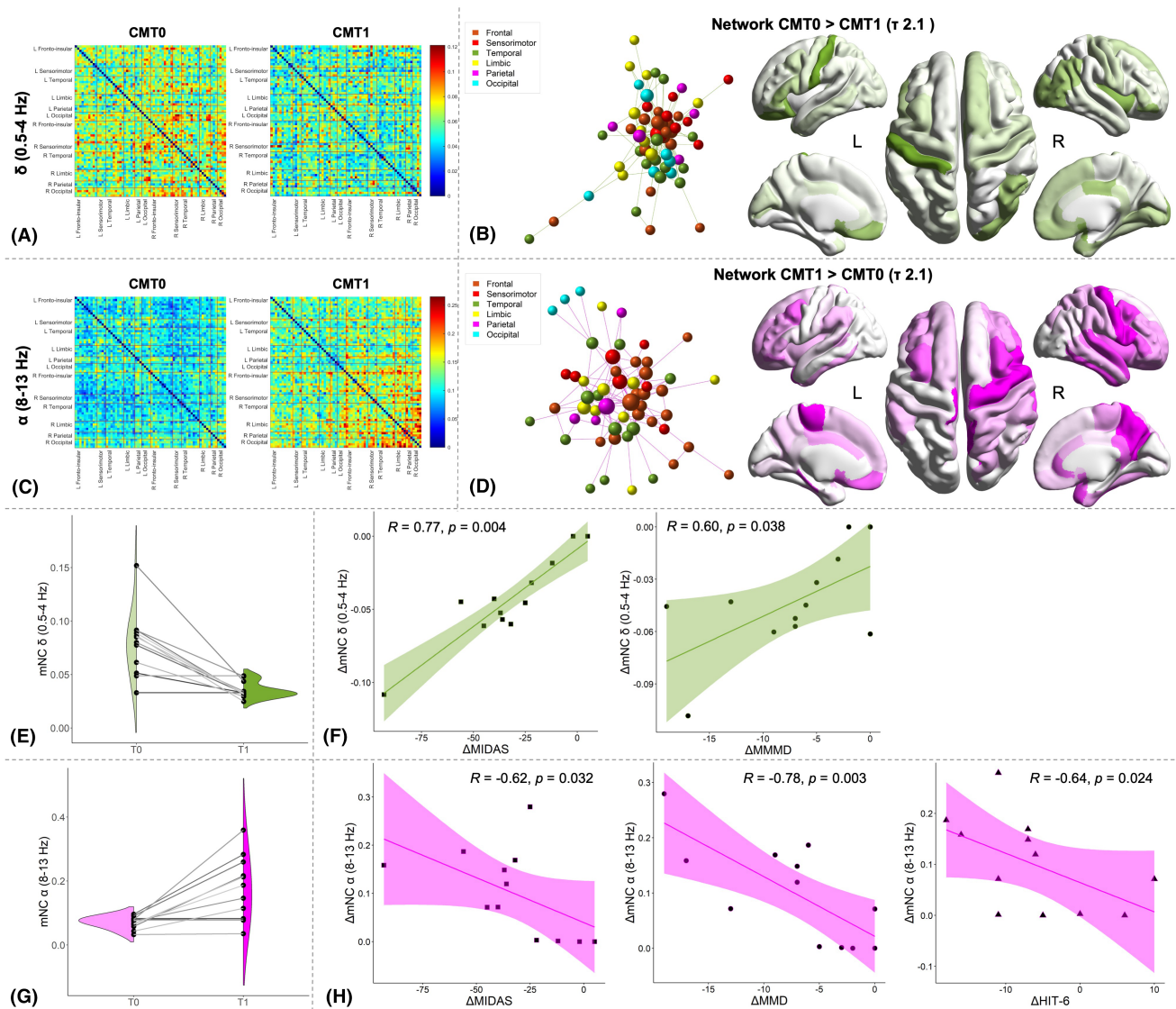


FIGURE 4 Mean FC matrices of CM-T1 and CM-T0 groups in δ (A) and α (C) bands. Differential network between CM-T0 and HC groups in δ (CM-T0 > CM-T1) (B) and α (CM-T1 > CM-T0) (D) bands. The left part of each panel shows graph representation of the differential networks. Colors denote different brain lobes: orange = fronto-insular, red = sensorimotor, yellow = limbic, green = temporal, magenta = parietal, light-blue = occipital; sphere diameters are directly proportional to region of interest (ROI) degrees. The right part of each panel represents ROIs degree of differential networks on model brain surface. Spaghetti plot of mean network connectivity (mNC) of the differential networks between CM-T1 and CM-T0 in δ (E) and α (G) bands. Significant Spearman's correlation between the Δ mNC of the differential networks and Δ value of the clinical scale across T1 and T0 points in in δ (F) and α (H) bands. CM-T0, chronic migraine at baseline; CM-T1, chronic migraine 3 months after treatment; FC, functional connectivity; HC, healthy control. [Color figure can be viewed at wileyonlinelibrary.com]

and right pars orbitalis), sensorimotor (mostly left paracentral and right precentral gyrus), temporal (mostly right temporal pole), and limbic (mainly bilateral anterior cingulate and right posterior cingulate) lobes, with prevalent connections in intra-frontal (17%), fronto-sensorimotor (11%), and fronto-temporal (10%) links (Figure 4C,D). Patient with CM mNCs of the α differential networks between CM-T1 and CM-T0 are shown in Figure 4G.

The reduction of mNC of δ network (τ 2.1) positively correlated with the reduction of MIDAS score ($r=0.77$, $p=0.004$, BS-95%CI [0.20 0.99]) and MMD ($r=0.60$, $p=0.038$, BS-95%CI [0.13 0.98]; Figure 4F). The increase of mNC of α network (τ 2.1) negatively correlated with the reduction of MIDAS ($r=-0.62$, $p=0.032$, BS-95%CI [-0.95 to 0.11]) and HIT-6 ($r=-0.78$, $p=0.003$, BS-95%CI [0.20 0.99]) scores, and MMD ($r=-0.64$, $p=0.024$, BS-95%CI [-0.93 to 0.11]; Figure 4H).

The Δ mNC of α network (τ 2.1) was significantly higher in responders compared to non-responders ($n=5$, Δ mNC 0.058 ± 0.028 ; $n=7$, Δ mNC 0.033 ± 0.026 , $p=0.048$). The Δ mNC of δ network (τ 2.1) was lower in responders compared to non-responders, although it didn't reach statistical significance ($n=5$, Δ mNC -0.075 ± 0.075 ; $n=7$, Δ mNC -0.14 ± 0.099 , $p=0.21$).

Cross-validation of NBS analysis

We analyzed the reliability and prediction performance of the NBS network found in the above analysis using a CV leave-one algorithm. Accuracy, precision, Cohen's κ , sensitivity, and specificity of the differential NBS network in CM-T0 versus HCs and CM-T1 versus CM-T0 conditions are reported in Table 2. ROC curves of predictive models are shown in Figure 5.

DISCUSSION

This pilot study investigated the functional features of patients with CM by means of HD-EEG and its modulation by OBTA treatment. We found that compared to HCs, patients with CM displayed functional alterations during RS in the interictal phase of migraine, which were network and band specific. In particular, we identified hyperconnected networks in δ and β bands, mainly involving orbitofrontal, occipital, temporal pole, and orbitofrontal, superior temporal, occipital, and anterior cingulate areas, and hypoconnected networks in α band, predominantly belonging to medium and posterior cingulate, temporal pole, and precuneus. The main finding of this study was a short-term modulation of FC in patients with CM following just a single session of OBTA treatment. Notably, patients with CM at T1, compared to T0, showed a hypoconnected network in the δ band and a hyperconnected network in the α band, which appeared mostly overlapping, both involving the sensorimotor, orbitofrontal, cingulate, and temporal pole cortex.

The design of our study allowed us to exclude several factors that could have influenced the results. First, there were no significant

TABLE 2 Cross-validation of the differential NBS network in CM-T0 versus HCs and CM-T1 versus CM-T0 conditions based on NBS predict leave-one-out method.

Networks	Accuracy	Precision	Cohen's κ	AUC	Sensitivity	Specificity
CM-T0 versus HC						
δ -CM-T0 > HC (τ 2.1)	0.91 [0.76 0.98]	0.91 [0.76 0.98]	0.80 [0.65 0.93]	0.91 [0.76 0.98]	0.83 [0.68 0.95]	0.95 [0.84 0.99]
α -HC > CM-T0 (τ 2.2)	0.85 [0.68 0.95]	0.71 [0.51 0.84]	0.70 [0.51 0.84]	0.94 [0.80 0.99]	1.00 [0.89 1.00]	0.76 [0.58 0.89]
β -CM-T0 > HC (τ 2.4)	0.82 [0.65 0.93]	0.75 [0.58 0.89]	0.61 [0.42 0.77]	0.85 [0.68 0.95]	0.75 [0.58 0.89]	0.86 [0.72 0.97]
CM-T1 versus CM-T0						
δ -CM-T1 > CM-T0 (τ 2.1)	0.88 [0.68 0.97]	0.80 [0.63 0.95]	0.75 [0.53 0.90]	0.89 [0.73 0.99]	1.00 [0.86 1.00]	0.75 [0.53 0.90]
α -CM-T0 > CM-T1 (τ 2.1)	0.79 [0.58 0.93]	1.00 [0.86 1.00]	0.58 [0.37 0.78]	0.78 [0.58 0.93]	0.66 [0.41 0.82]	1.00 [0.86 1.00]

Note: The parameters reported are in the first row the value of accuracy, precision, Cohen's kappa, AUC, sensitivity and specificity, and in the second row their relative 95% confidence interval (95%CI). Abbreviations: AUC, area under the receiver operating characteristic curve; CM-T0, chronic migraine at baseline; CM-T1, chronic migraine 3 months after treatment; HC, healthy control; NBS, network-based statistic.

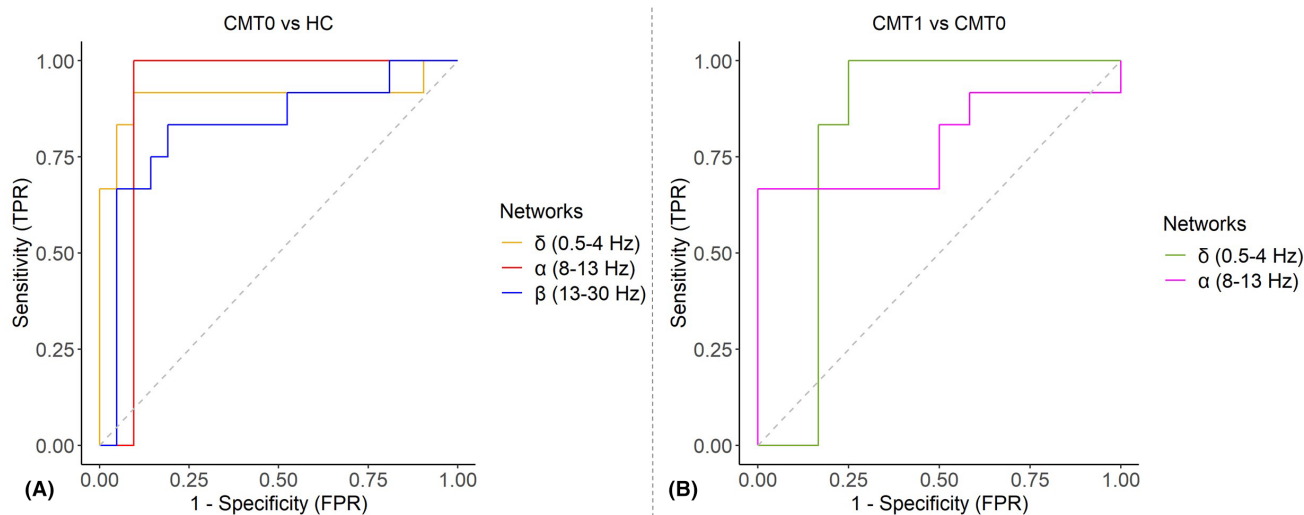


FIGURE 5 ROC curve based on NBS predict method of the differential networks between CM-T0 and HC (A) and CM-T1 and CM-T0 (B). CM-T0, chronic migraine at baseline; CM-T1, chronic migraine 3 months after treatment; HC, healthy control; NBS, network-based statistics; ROC, receiver operating characteristic. [Color figure can be viewed at wileyonlinelibrary.com]

differences between patients with CM and HCs in age or sex, and the analyses were also covariate for these potential confounding factors. Indeed, both age and sex are well-known factors affecting FC.⁴¹ Likewise, all the analyses were performed during the inter-ictal phase of migraine to identify chronic changes in the FC of CM, independent of the single attack.

Previous studies, mostly based on fMRI, have found significant alterations during resting state in FC between cortical regions involved in the processing of pain and pain-related cognitive and emotional experiences, referred to as the “neurolimbic pain network.”^{42,43} The primary nodes of this network include the sensorimotor cortex, involved in the processing of pain; the cingulate and amygdala, which mostly involve affective and emotional processing; the orbitofrontal cortex, which controls the cognitive processing of pain; the temporal pole, precuneus, and hippocampus, which play a key role in emotion memory; and several subcortical and brainstem structures.¹⁴

In line with these findings, we found a disrupted FC in chronic migraine patients based on HD-EEG, mostly involving cortical areas implicated in the processing of pain. In these patients, the limbic lobe (anterior and posterior cingulate) was hyperconnected in delta and beta and hypoconnected in the alpha band. In contrast, the orbitofrontal area was hyperconnected in delta and beta bands, confirming the important role of altered conscious pain processing in migraine. Moreover, the results of the present study confirmed an alteration in sensorimotor areas in patients with CM, which resulted in being hyperconnected in delta and beta bands, mirroring a disruption in multisensory integration, nociception processing, spatial attention, and intention.⁴⁴

As mentioned above, the alterations found in EEG-FC appeared to be not only network, but also band specific. Literature investigating EEG-based FC in CM is relatively scarce, while many studies have examined the spectral properties of quantitative EEG in CM in inter-ictal or ictal phases.^{45–47} δ power was shown to be slightly

increased in the fronto-central regions in patients with CM in the interictal phase.⁴⁸ Moreover, previous research, although based on episodic migraine patients, suggests that migraine is characterized during the inter-ictal phase by an increase in δ power ipsilateral to the side of the head, where pain is most prominent during headache attacks, possibly a marker of a latent cortical spreading depression.⁴⁹ Accordingly, our patients with CM presented diffuse hyperconnectivity in the δ band compared to controls. Furthermore, the altered FC in the δ band correlated with CM disability as assessed through the MIDAS score. Here, we did not find a lateralized alteration but rather a diffuse, bi-hemispherical hyperconnectivity that significantly correlated with the overall CM-related burden. Hence, migraine patients with greater disability seem to display slower activity in all cortical areas.

Moreover, migraine research has consistently found evidence of electrophysiological abnormalities of α activity.^{50,51} The α rhythm is related to functional inhibition of the sensory cortex and might represent the GABAergic inhibitory feedback paced by neocortical or thalamic rhythm generators.⁵² Thus, we observed a disrupted FC in the α band that is consistent with a cortical hyper-responsivity typically found in migraine^{14,53} as an expression of an underlying “thalamocortical dysrhythmia,”⁵⁴ in which a deficient thalamocortical drive leads to a dysfunction of both inhibitory and excitatory cortical neurons.⁵⁵ Furthermore, some studies supported the hypothesis that α -FC disruption is related to the involvement of ascending and/or cortical cholinergic systems in neurodegenerative disorders.^{56,57} A recent study by Coppola et al. found that cholinergic facilitatory thalamocortical activation is reduced in migraine during the inter-ictal phases but increased ictally.⁵⁸

The main finding of the present study was that a single OBTA treatment session resulted in a short-term modulation of altered δ and α FC in patients with CM. Notably, the significant differences in FC

found at baseline between patients with CM and controls were lost just 3 months after the start of OBTA treatment. These data align with prior studies finding a rapid neuromodulator effect of botulinumtoxin-A (BTA) on the nociceptive networks at the central level.¹⁵

These FC changes matched a clinical improvement in terms of both the number of days of migraine and overall disability, as measured by the MIDAS scores and the HIT-6 scale, although these electrophysiological correlations might reflect the improvement in migraine-related disability rather than a specific OBTA effect. Indeed, these correlations with functional changes align with previous fMRI studies that have reported similar results in the context of migraine-related disability improvement as a consequence of preventive therapy.⁵⁹

The efficacy of OBTA for CM treatment was first established in the PREEMPT trials.⁶ Several real-life studies confirmed the role of OBTA in reducing the impact of CM on multiple aspects, such as frequency, disability, and pain intensity.⁵ Accordingly, we found that just a single cycle of OBTA treatment was able to significantly reduce the overall migraine-related burden, further supporting the speed of action of OBTA.⁶⁰ Nevertheless, the way BTA might induce central changes in CM is still debated. While animal studies provide evidence for retrograde transport of BTA,⁶¹ retrograde transport has not been clearly assessed in humans,⁶² and the current literature suggests that the central effects of BTA on the brain may be predominantly indirect.⁶³

Individuals affected by CM exhibit a persistent peripheral sensitization, which ultimately gives rise to central sensitization, facilitating pain persistence and leading to functional changes within the nociceptive system.^{14,64} Clinical correlates of the central sensitization phenomena are so-called allodynia (pain induced by stimuli that typically do not cause pain) and hyperalgesia (heightened sensitivity to pain).⁶⁵

OBTA, reversibly reducing the exocytosis of neuropeptides and neurotransmitters from peripheral sensory neurons, temporarily breaks this vicious cycle, possibly leading to a reduction of the phenomenon of central sensitization.⁶⁶ Therefore, the reversion of peripheral sensitization might result in modulation and plastic reorganization within the CNS.⁶³ Whether previous findings already showed that long-term toxin treatment could modulate phenomena of central sensitization and reduce migraine disability,⁶⁵ this was the first study to assess such rapid changes at central levels. These findings further suggest the primary role of the central mechanisms of OBTA, although probably indirect, in the treatment of CM, which might be even more important than the peripheral mechanisms of migraine attack prevention.

It is well known that phenomena of central sensitization correlate with mood and sleep disturbances.⁶⁷ Here, we did not find a significant effect of OBTA treatment on anxiety or depression features, as assessed through the BDI and HADS questionnaires. However, it is possible that an effect on specific neuropsychiatric manifestations would require a longer duration of treatment, as previous reports suggested.⁶⁸

Finally, we found that β connectivity correlated with pain intensity as assessed through the VAS scale; however, neither β

connectivity nor VAS scores were affected by OBTA treatment. Nevertheless, it is possible that a longer duration of treatment would have been necessary to modulate pain intensity over time.

Although it allowed enlightenment of the rapidity of central changes within the CNS induced by OBTA treatment, this study has several limitations and only preliminary results. The main limitations of this study are certainly the small number of patients included and the relatively short follow-up period. Indeed, although NBS has the power to detect network alterations, its results are characterized by relatively poor stability, mainly if the analyses are performed in a small cohort.

Therefore, we used a CV method that showed the analysis's reliability and prediction performance. Nevertheless, we know that CV cannot replace the analysis performed on a larger patient cohort and/or an external validation sample, which are necessary to confirm these results.

Another limitation of this study is the lack of placebo control, as placebo is known to significantly weight on the therapeutic effect in migraine.⁶⁹ Nonetheless, the present study deliberately opted not to include a placebo control, given the burdening symptomatology experienced by the participating patients. Moreover, we did not restrict patients from taking acute medications during the study, although it could represent a potential confounding factor. No patient reported medication overuse, and the FC analysis was not altered from acute medications because each EEG recording was performed at least 72h after taking acute therapy.

CONCLUSIONS

This pioneering study found that OBTA treatment had a rapid and prompt action on FC that matched the beneficial clinical effects. Further studies, including larger cohorts of patients and longer follow-up periods, should be performed to validate our findings and provide a more comprehensive understanding of the central effects of OBTA treatment.

AUTHOR CONTRIBUTIONS

Matteo Conti: Conceptualization; data curation; formal analysis; methodology; software; writing – original draft. **Roberta Bovenzi:** Conceptualization; data curation; investigation; methodology; writing – original draft. **Maria Giuseppina Palmieri:** Supervision; validation. **Fabio Placidi:** Resources; validation. **Alessandro Stefani:** Funding acquisition; resources; validation. **Nicola Biagio Mercuri:** Supervision; validation; writing – review and editing. **Maria Albanese:** Conceptualization; supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Matteo Conti, Roberta Bovenzi, Maria Giuseppina Palmieri, Fabio Placidi, Alessandro Stefani, Nicola Biagio Mercuri, and Maria Albanese declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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