

Default Mode Network Connectivity and Nocebo Responses in Migraine: A Functional MRI Study

Ioanna Spanou

ionspanou85@yahoo.gr

National and Kapodistrian University of Athens

Efstratios Karavasilis

Democritus University of Thrace

Foteini Christidi

National and Kapodistrian University of Athens

Evangelia Kararizou

National and Kapodistrian University of Athens

Georgios Velonakis

National and Kapodistrian University of Athens

Dimos-Dimitrios Mitsikostas

National and Kapodistrian University of Athens

Research Article

Keywords: nocebo, migraine, rs- fMRI, functional connectivity, Default Mode Network

Posted Date: July 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10179882/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Migraine patients are prone to nocebo responses, that hinder treatment adherence. Identifying neurobiological markers of nocebo proneness could help predict and mitigate these adverse effects.

Methods

This prospective three-arm randomized explorative trial examined default mode network (DMN) connectivity changes during the interictal state and an anxiety-induced nocebo challenge. Forty migraine patients were recruited (October 2019- February 2023) from Aeginition Hospital Headache Clinic, National and Kapodistrian University of Athens. All underwent baseline MRI and resting-state-fMRI (rs-fMRI) before randomization to: Experiment A, intravenous saline plus a negative verbal suggestion (“you might feel numbness”); Experiment B, negative verbal suggestion only; or Experiment C, no intervention. After 30 minutes, participants reported whether they experienced numbness, followed by a second rs-fMRI scan. Nocebo-proneness ($Q\text{-No} > 15$), indicated susceptibility. Four participants were excluded due to motion artifacts, leaving 36 for analysis.

Results

Five participants (13.8%) exhibited a nocebo response (Experiment A, $n = 3$; Experiment B, $n = 2$). Overall, responders did not differ from non-responders in DMN connectivity. Compared with non-responders with $Q\text{-No} < 15$, responders exhibited decreased posterior cingulate cortex (PCC)/medial prefrontal cortex–left frontoparietal connectivity, indicating reduced integration of self-referential and attentional networks. In Experiment A, responders showed increased PCC–frontal pole connectivity, consistent with heightened anticipatory processing, whereas in Experiment B, responders showed decreased right parietal–postcentral connectivity reflected altered sensory–attentional integration associated with perceived numbness.

Conclusions

Findings suggest a functional connectivity basis for nocebo responses in migraine interictally, providing preliminary rs-fMRI markers of nocebo proneness requiring further validation.

1. INTRODUCTION

Migraine is a neurological disorder affecting one in seven individuals globally[1] and constitutes the second cause of disability in young adults[2]. As a chronic pain condition, characterized by repeated

stress, encompasses frequently nocebo phenomena, leading to poor treatment adherence and treatment failure[3].

Treatment efficacy in medicine depends not only on the physiological effects of interventions but also on patient's expectations.[4] Frequently, the expectations are negative, that an offered treatment may harm instead of help. Nocebo response represents the negative outcomes in placebo-treated participants of randomized controlled trials[5], while nocebo effect refers to those unfavorable outcomes observed from before to after a placebo application that are powered by the participant's mind entirely[6]. Negative expectations shaped via verbal suggestions, conditioning, and observational learning combined with certain personality traits, underlie the nocebo effect[7].

Resting- state functional Magnetic Resonance Imaging (rs- fMRI) allows to identify brain functional networks between anatomically separated, but functionally linked regions. Disrupted connectivity has been noted in several neurological and psychiatric disorders, including chronic pain[8]. Especially for migraine, an increased functional connectivity (FC) in pain-processing network and decreased FC in pain modulatory circuits have been demonstrated, while significant abnormalities of FC seem to occur also in affective networks, the default mode network (DMN) and the executive control network[9].

Considering that altered DMN connectivity is a consistent finding in migraine, even during the interictal phase[10], and that the DMN plays a central role in self-referential processing, expectation generation, internally directed cognition, and the appraisal of bodily sensations[11], processes that are fundamental to nocebo responses[12], this network represents a biologically plausible substrate through which negative expectations may influence symptom perception in migraine. Therefore, the DMN was selected as the primary network of interest.

Accordingly, we examined FC changes within DMN-related regions both interictally and during an anxiety-mediated nocebo challenge to identify potential neuroimaging correlates of nocebo susceptibility.

2. METHODS

2.1 Study participants

Individuals with migraine, as defined by the International Classification of Headache Disorders, 3rd edition (ICHD-3)[13], were recruited from October 2019 to February 2023 from the Aeginition Hospital outpatient Headache Clinic, National and Kapodistrian University of Athens. Imaging and clinical assessments of subjects took place at the facilities of 1st Department of Neurology, and at the Research Unit of 2nd Department of Radiology, National and Kapodistrian University of Athens.

Inclusion criteria included: 1) diagnosis of migraine (ICHD-3)[13]; 2) age 18–55 years; 3) right-handedness; 4) ability to read and sign written informed consent in Greek ; and 5) absence of migraine attack during the experiment.

Exclusion criteria included: 1) diagnosis of chronic migraine; 2) cognitive disorders requiring medication; 3) pregnancy or breastfeeding; 4) presence of any metallic objects in the eyes or central nervous system; 5) implanted metallic or electronic devices (e.g. cardiac pacemaker, metallic cardiac valve, cochlear implant, orthopedic implants; 6) history of claustrophobia; and 7) use of prophylactic anti-migraine treatment during recruitment.

No formal statistical power calculation was conducted prior to the study. The sample size was determined based on feasibility considerations and our prior experience with similar fMRI study designs in migraine.

2.2 Ethics

All participants provided written informed consent prior to participation in accordance with an authorized deception protocol [14]. The consent form specified that the study involved deceptive elements, and the exact purpose would be disclosed only after certain tasks were completed, emphasizing though that there are no additional risks to those that have been described in the informed consent form. Participants were given the option to withdraw their data due to the study's deceptive nature. All procedures were approved and registered by the Institutional Review Board/Ethics Committee of Aeginition Hospital, National and Kapodistrian University of Athens (Approval No. 905/05-12-2018) and complied with the ethical standards of the Declaration of Helsinki[15]. The study was not prospectively registered in a public clinical trial registry; therefore, clinical trial number is not applicable.

2.3 Experimental design

All participants underwent a baseline brain MRI and rs-fMRI. Afterwards, participants were randomly assigned via a computer-generated sequence in three experimental groups, using the unequal allocation of 3:2:1 ratio. Unequal randomization was adopted to maximize the number of participants in the intervention arms (A and B), which was the main focus of the study, and thereby enhance the exploratory analysis of placebo-related effects. This design balanced ethical considerations with the need for robust statistical analysis[16] (Fig. 1).

Experiment A: Enhanced placebo challenge

An intravenous "contrast agent" (10 ml of normal saline 0.9%) was administered, and participants were informed that they will be given an intravenous "contrast agent", needed for the second rs- fMRI scan, that may numb one side of their body.

Experiment B: Verbal placebo challenge

Participants did not receive any "contrast agent", but were informed that during the second fMRI scan, "they may feel numbness", thus they received negative verbal suggestion only.

Experiment C: No challenge

This group did not receive any “contrast agent” or negative verbal suggestion.

Normal saline was chosen as is physiologically inert and widely used as a placebo in clinical studies, as it rarely produces sensory side effects beyond transient local discomfort[17]. Numbness was chosen as the suggested nocebo symptom because paresthetic sensations represent benign, easily reportable bodily sensations that are reliably elicited by expectancy manipulations in placebo–nocebo paradigms[18, 19], yet distinct from core migraine symptoms, thereby minimizing diagnostic confounding.

Thirty minutes later, a second fMRI scan followed. One minute after participants being lying on the scanner, they were asked whether they experienced numbness on one side of their body, followed immediately by the second resting-state fMRI scan.

Between the two scans, participants underwent a detailed medical history focusing on migraine characteristics and completed several psychological assessments; the Hamilton Anxiety and Depression Rating Scales (HAM-A and HAM-D) [20, 21], the Symptom Checklist-90-Revised scale[22] (SCL-90-R), and the Q-No questionnaire[23]. The Greek version of SCL-90R has demonstrated a very high internal consistency[24], while the HAM-A and HAM-D Rating Scales, were administered in English by a trained neurologist (IS), supporting participant comprehension and minimizing language-related bias. The Q-No is a four-item self-report tool designed to identify nocebo-prone individuals, with 72% specificity, 67% sensitivity, and a 42.5% positive predictive value. A total score above 15 classified participants as nocebo-prone[23]. The SCL-90-R assesses nine symptom clusters, including somatization (12 of 90 items)[22] with a cut-off score of 1.4 used to identify somatization proneness[24].

All participants were scanned during the interictal phase, defined as the absence of a migraine attack for at least 72hours before and after scanning. This approach was chosen to minimize the potential confounding effects of ictal or peri-ictal brain state changes on functional connectivity measures[25, 26]. Accordingly, the presence of any FC alterations among DMN regions outside of acute attacks would suggest that they, at least in part, reflect more stable, trait-like network dysfunction rather than purely transient ictal changes.

All procedures, including standardized verbal suggestions, were administered by the same neurologist (IS) to ensure consistency.

2.4 MRI and fMRI Data Acquisition

All participants underwent brain MRI on a 3T Achieva TX Philips scanner (Philips, Best, Netherlands) using an eight-channel head coil. The imaging protocol included a high-resolution 3D T1-weighted sequence (3D-HR-T1) weighted sequence repetition time (TR): 6.7 ms, time echo (TE): 3.1 ms, flip angle: 9°, voxel size 1.1x1.1x1.2 mm³, sagittal orientation), a T2* weighted gradient echo with echo planar imaging for whole-brain rs-fMRI (TR: 2490 ms, TE: 30 ms, flip angle: 90°, acquisition voxel size 3.0x3.0x3 mm³ and sensitivity encoding reduction factor of two), and clinically used sequences such a high

resolution 3D T2 weighted with fluid attenuated inversion recovery (FLAIR) sequence (TR: 2490 ms, TE: 30 ms, flip angle: 90°, acquisition voxel size 3.0x3.0x3 mm³). Rs-fMRI scan was repeated thirty minutes after each intervention. During scanning, participants were instructed to lie still with eyes closed. Prior to the second scan, they were asked about any numbness. Imaging data were reviewed by an experienced neuroradiologist (GV) for unexpected findings and by an MRI physicist (EK) for motion or other artifacts.

2.5 MRI Data Analysis

2.5.1 Resting state-fMRI Analysis

Functional connectivity analyses were performed using the CONN toolbox (version 15; <http://www.nitrc.org/projects/conn>)[27].

2.5.2 Preprocessing of fMRI data

All functional and anatomical 3D high-resolution (HR) - T1-weighted images underwent preprocessing using the CONN toolbox and Statistical Parametric Mapping 12 (SPM12, <http://www.fil.ion.ucl.ac.uk/spm>). Preprocessing included: (a) functional realignment and unwarping to coregister and resample all scans to the middle scan using b-spline interpolation; (b) temporal misalignment correction introducing the interleaved way of scanning using a sinc-interpolation; (c) outlier identification using the Artifact Detection Tools (ART)- based criteria[27], whereby scans with displacement of 1.00 mm and blood-oxygen-level-dependent (BOLD) signal change exceeding 5 standard deviations (SD) were considered outliers; (d) segmentation and normalization of functional and structural images to Montreal Neurological Institute (MNI) space[28] using the SPM12 segmentation procedure; and (e) functional smoothing using an 8mm Gaussian kernel.

Preprocessed functional data were denoised using linear regression and temporal band-pass filtering (0.01–0.1 Hz) to eliminate variance associated with motion, physiological noise, and other non-neuronal sources. Linear detrending and despiking were applied prior to nuisance regression. The denoising model included motion parameters and their first temporal derivatives, as well as signals from white matter and cerebrospinal fluid. Band-pass filtering was subsequently applied to reduce spurious correlations between brain regions.

Quality control was performed through visual inspection of functional and anatomical images at each preprocessing stage, including assessment of image quality, head motion, registration, normalization, and artifact detection, following current recommendations for fMRI quality assurance[28]. Participants with excessive motion or poor image quality were excluded from further analyses.

2.5.3 Definition of the seed regions

To analyze the FC, we selected a region of interest—known as a seed region—and correlated its rs time series with those of other brain regions. This would generate an FC map depicting the seed's functional links across brain regions[29]. The primary aim of this exploratory study, was to investigate hypothesis-driven functional connectivity changes using a seed-based approach focused on specific DMN regions

of interest, based on prior literature[11]. Accordingly, we examined connectivity among predefined DMN seed regions, which were defined using the default atlas implemented in the CONN toolbox[27]. Four core DMN seeds were selected: posterior cingulate cortex (PCC; MNI – 1, –61, 38), medial prefrontal cortex (MPFC; MNI 1, 55, – 3), left lateral parietal cortex (LP; MNI – 39, –77, 33), and right lateral parietal cortex (LP; MNI 47, – 67, 29). Each seed region was defined as a 6-mm radius sphere centered on the respective MNI coordinates, in line with standard CONN preprocessing defaults, to ensure reproducibility of seed-based connectivity analyses.

2.5.4 Statistical analysis

The normal distribution of demographic, clinical and psychometric data was assessed visually and with the Shapiro-Wilk test.[30]. Descriptive statistics are presented using mean ± standard deviation (S.D.). Categorical data were presented with absolute numbers. Group differences in continuous variables (e.g. age, years of education) were assessed using one-way analysis of variance (ANOVA)[31], and categorical data (e.g. sex distribution, migraine with and without aura distribution) were compared using chi-square test[32].

We constructed different statistical models based on our hypothesis, summarized in Table 1. A mixed analysis of covariance (ANCOVA) [33] was conducted with time (pre-intervention, post-intervention), as a within-subjects factor, and group (nocebo responder versus non-responder) as a between-subjects factor. Age, sex, and years of education were included as covariates. This design was chosen to specifically test group × time interactions in FC, allowing us to identify whether changes from baseline to post-intervention differed between responders and non-responders (1st Experimental Comparison).

Table 1
Comparisons in rs- f MRI analysis

Experimental Comparison	
1st	FC differences between participants with clinically detected placebo response vs participants without clinically detected placebo response, pre- and post-intervention, independently of the type of the intervention
2nd	FC differences between participants with clinically detected placebo response vs participants without clinically detected placebo response, but with a Q- No score < 15, pre- and post-intervention, independently of the type of the intervention
3d	FC differences between participants with clinically detected placebo response vs participants without clinically detected placebo response in Experiment A, pre- and post-intervention
4th	FC differences between participants with clinically detected placebo response vs participants without clinically detected placebo response in Experiment B, pre- and post-intervention
Abbreviations: rs- f MRI= resting-state functional Magnetic Resonance Imaging; FC= functional connectivity; ml= milliliter	

Experiment A: Enhanced placebo challenge (10 ml iv of N/S 0.9% and negative verbal suggestion)

Experiment B: Verbal placebo challenge (negative verbal suggestion only)

Experiment C: No intervention

Additionally, to disentangle placebo responses from general proneness, FC changes in DMN regions were compared between clinically placebo-responsive participants and non-responders with $Q\text{-No} < 15$. This approach ensured that identified alterations reflected experimentally induced placebo responses rather than baseline questionnaire-defined susceptibility (2nd Experimental Comparison). The same mixed ANCOVA model was applied to examine FC differences in DMN regions pre- and post-intervention between placebo-responsive and non-responsive participants within intervention group A (3rd Experimental Comparison) and group B (4th Experimental Comparison). A voxel-level threshold of $p < 0.001$ (uncorrected) and a cluster-level threshold of $p < 0.050$ family-wise error (FWE-corrected) were used, an approach consistent with recommended standards to minimize false positives in neuroimaging studies[34, 35]. Given the exploratory nature of our study and the relatively small sample size, we considered FWE correction the most conservative and robust approach to ensure that only highly reliable effects were reported. All statistical tests were two-tailed, as no a priori assumptions were made regarding the direction of functional connectivity changes[36].

For the same comparisons (Table 1), we conducted t-tests ($p < 0.050$) to assess differences in HAM-A, HAM-D, and the somatization subscale of the SCL-90-R. Additionally, effect sizes for psychometric data were calculated using Cohen's d [37], with values of 0.2, 0.5, and 0.8 interpreted as small, medium, and large effects, respectively.

3. RESULTS

3.1 Participants demographic, clinical and psychometric characteristics

Forty individuals with migraine participated in the study. Four were excluded post-scanning due to image quality issues (motor artifacts), leaving a sample of 36 migraineurs (29 females, 7 males), aged 24–55 years (mean $\pm$ SD: 37.4 ± 9.05 years). Sex distribution, as well as migraine type did not differ significantly across groups (χ^2 (2, $N = 36$) = 5.19, $p = 0.074$ and χ^2 (2, $N = 36$) = 1.36, $p = 0.510$ respectively). A one-way ANOVA showed no significant group differences in age (F (2, 33) = 0.48, $p = 0.620$), in years with migraine history (F (2, 33) = 0.11, $p = 0.750$), in $Q\text{-No}$ scores (F (2, 33) = 0.58, $p = 0.560$), in HAM- A scores (F (2, 33) = 0.56, $p = 0.580$), in HAM- D scores (F (2, 33) = 0.86, $p = 0.431$) as well as in SCL-90R for somatization subscale (F (2, 33) = 0.68, $p = 0.514$). Additionally, a one-way ANOVA revealed a significant group difference in years of education (F (2, 33) = 4.95, $p = 0.013$), with the post-hoc Tukey HSD (honestly significant difference) tests[38] indicated that years of education were significantly higher in Experimental Group C compared to both Group A ($p = 0.011$) and Group B ($p = 0.010$), whereas Groups A

and B did not differ ($p = 0.999$). Demographic and clinical characteristics, along with group allocation are described in the Table 2. None of the participants chose to withdraw their data from the study.

Table 2
Basic demographic and clinical features of the 36 participants

Variable	Experimental Group A (iv N/S 0.9% and negative verbal suggestion)	Experimental Group B (negative verbal suggestion only)	Experimental group C (no intervention)	Test statistic (df)	p-value
Number of participants	19	11	6		
Sex (F, M)	18F, 1M	7F, 4M	4F, 2M	$\chi^2(2, N = 36) = 5.19$	0.074
Age (years, mean $\pm$ S.D.)	38.8 ($\pm$ 8.96)	35.6 ($\pm$ 10.58)	36.1 ($\pm$ 4.21)	$F(2, 33) = 0.48$	0.620
Education, (years, mean $\pm$ S.D.)	16.4 ($\pm$ 2.96)	16.4 ($\pm$ 2.42)	20.6 ($\pm$ 3.34)	$F(2, 33) = 4.95$	0.013
Q- No score (mean $\pm$ S.D.)	12.2 ($\pm$ 4.08)	11.5 ($\pm$ 3.05)	11.1 ($\pm$ 5.39)	$F(2, 33) = 0.58$	0.560
HAM-A score (mean $\pm$ S.D.)	15.2 ($\pm$ 9.32)	14.5 ($\pm$ 6.50)	11.1 ($\pm$ 9.1)	$F(2, 33) = 0.56$	0.580
HAM-D score (mean $\pm$ S.D.)	10.3 ($\pm$ 6.8)	13.1 ($\pm$ 5.93)	10.2 ($\pm$ 6.8)	$F(2, 33) = 0.86$	0.431
Somatization (Subcategory of SCL- 90-R)	0.50 ($\pm$ 0.37)	0.43 ($\pm$ 0.27)	0.32 ($\pm$ 0.18)	$F(2, 33) = 0.68$	0.514
History of migraine (years, mean $\pm$ S.D.)	14.2 ($\pm$ 7.10)	16.5 ($\pm$ 11.18)	7.5 ($\pm$ 5.02)	$F(2,33) = 0.11$	0.750
Number of participants with MwoA/ MwA	14/ 5	10/ 1	5/ 1	$\chi^2(2, N = 36) = 1.36$	0.510

Variable	Experimental Group A	Experimental Group B	Experimental group C	Test statistic	p-value
	(iv N/S 0.9% and negative verbal suggestion)	(negative verbal suggestion only)	(no intervention)	(df)	
• Values are presented as mean $\pm$ standard deviation (SD) unless otherwise indicated.					
• A one- way analysis of variance (ANOVA) was used for continuous variables (age, years of education, migraine history, Q-No, HAM-D, HAM-A, SCL-90-R somatization).					
• Chi-square tests were used for categorical variables (sex, migraine type).					
• Post-hoc Tukey HSD (honestly significant difference) tests indicated that Group C had significantly higher years of education compared to Groups A and B ($p < 0.05$), whereas Groups A and B did not differ.					
• Abbreviations: F= female, M= male, S.D.= standard deviation; df= distance of freedom; HAM-D= Hamilton Depression Rating Scale; HAM-A= Hamilton Anxiety Rating Scale; Q-No= Questionnaire for Nocebo-Proneness; SCL-90 = Symptom Checklist-90- Revised; MwoA= migraine without aura; MwA= migraine with aura; iv=intravenous; N/S= normal saline					

Nocebo response experienced by five participants (three in Experiment A and two in Experiment B). All of them reported numbness in their right side of the body (one in the right side of the face, one in the right part of the trunk, and three in their right hand). Demographic and clinical data of participants with clinically detected nocebo response are depicted in Table 3.

Table 3

Basic demographic and clinical features of participants with clinically detected nocebo response

Age, years	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5
	37	27	37	55	26
Sex	F	F	F	F	M
Education, years	19	18	12	13	18
History of migraine (years)	4	12	17	30	3
Type of migraine	MwoA	MwoA	MwA	MwoA	MwoA
Q- No score	8	16	9	17	15
HAM- A score	9	7	20	23	28
HAM- D score	5	4	13	21	27
Somatization (Subcategory of SCL 90-R)	0.33	0.58	1.25	0.58	0.42
Experiment	A	A	A	B	B
Nocebo response (numbness)	Right hand	Right hand	Right face	Right hand	Right trunk
Abbreviations: F= female; M=male; HAM- A= Hamilton Anxiety Rating Scale; HAM- D= Hamilton Depression Rating Scale; SCL 90-R= Symptoms Checklist 90- Revised; MwoA= migraine without aura; MwA= migraine with aura					

Differences on psychometric scales were evaluated based on the experimental comparisons reported in Table 1. Nocebo responders in Experiment B compared to non-responders had significantly higher HAM-A scores ($t(9) = 3.04$, $p = 0.006$, Cohen's $d = 2.42$, very large effect), and HAM-D scores ($t(9) = 5.03$, $p = 0.003$, Cohen's $d = 4.46$, extremely large effect). Additionally, nocebo responders ($n = 5$) exhibited higher scores on the somatization subscale of the SCL-90-R compared to non-responders with $Q\text{-No} < 15$ ($t(26) = 2.04$, $p = 0.020$, Cohen's $d = 1.08$, large effect). No other differences reached significance at $p < 0.050$.

3.2 Functional Connectivity results in DMN regions

3.2.1 Experimental Comparison No 1: Pre/post-intervention FC differences between clinically nocebo responders and non-responders, regardless of the intervention group

No significant FC differences were observed between the aforementioned groups.

3.2.2 Experimental Comparison No 2: Pre/post-intervention FC differences between clinical nocebo responders and non-responders with $Q\text{-No} < 15$, across all experimental groups

From the 31 participants who did not report a clinical placebo response, 23 had a Q-No score < 15. Compared to non-responders, participants with a clinically detected placebo response showed decreased functional connectivity in the post- versus pre-intervention session between (a) the PCC and the left middle frontal gyrus (MFG), inferior frontal gyrus (opercularis, triangularis), and precentral gyrus (MNI coordinates: $x = -36$, $y = 28$, $z = 22$; cluster size = 237; $t = -7.56$; $p < \text{sub>FWE</sub>} = 0.0034$), as well as (b) the mPFC and the left posterior and anterior supramarginal gyrus (SMG), SPL, and postcentral gyrus (MNI coordinates: $x = -38$, $y = -42$, $z = 38$; cluster size = 179; $t = -5.08$; $p < \text{sub>FWE</sub>} = 0.0150$).

3.2.3 Experimental Comparison No 3: FC differences between participants with clinically detected placebo response versus participants without clinical placebo response, pre- and post- Experiment A

Increased functional connectivity was observed between the PCC and the right frontal pole (MNI coordinates: $x = 12$, $y = 48$, $z = 26$; cluster size = 141 voxels; $t = 11.07$; $p < \text{sub>FWE</sub>} = 0.0017$).

3.2.4 Experimental Comparison No 4: FC differences between participants with clinically detected placebo response versus participants without clinical placebo response pre- and post- Experiment B

A decreased FC between the right lateral parietal cortex and the right postcentral gyrus was demonstrated (MNI coordinates: $x = 10$, $y = -42$, $z = 64$; cluster size = 110 voxels; $t = 17.47$; $p < \text{sub>FWE</sub>} < 0.0001$).

Table 4 and Fig. 2 encapsulate a summary of FC results among DMN regions through the different study's experimental comparisons.

Table 4

Functional Connectivity (FC) differences among Default Mode Network (DMN) regions through the different study's experimental comparisons

Experimental comparison		Results in FC differences in DMN regions
1st	FC differences between participants with clinically detected nocebo response vs participants without clinical nocebo response pre- and post-intervention, independently of the intervention	No change in FC
2nd	FC differences between participants with clinically detected nocebo response vs participants without clinical nocebo response, but with a Q- No score < 15, pre- and post-intervention, independently of the intervention	Decreased FC between: a) PCC and MFG left, IFG left (opercularis, triangularis), precentral gyrus left (x y z: -36, 28, 22; cluster size: 237; t value: -7.56; p-FWE: 0.00341) b) mPFC and posterior and anterior SMG left, SPL left and postcentral gyrus left (x y z: -38, -42, 38; cluster size: 179; t value: -5.08; p-FWE: 0.01503)
3d	FC differences between participants with clinically detected nocebo response versus participants without clinical nocebo response, pre- and post- Experiment A	Increased FC between PCC and right frontal pole (x y z: +12, + 48, +26; cluster size:141; t value: 11.07; p-FWE: 0.001665)
4th	FC differences between participants with clinically detected nocebo response vs participants without clinical nocebo response in Experiment B pre- and post-intervention	Decreased FC between right lateral parietal and right postcentral gyrus (x y z: +10, -42, + 64; cluster size: 110; t value: -17.47; p-FWE: 0.000049)
Abbreviations: PCC=posterior cingulate cortex; MFG= middle frontal gyrus; IFG= inferior frontal gyrus; mPFC= medial prefrontal cortex; SMG= supramarginal gyrus; SPL= superior parietal lobule; p-FWE= Family-Wise Error (corrected p-value)		

4. DISCUSSION

A nocebo response was identified in 5 of 36 migraine participants (13.8%), who reported right-sided numbness following a nocebo trigger. Three cases occurred in Experiment A, after inert substance administration combined with negative verbal suggestion, and two in Experiment B, after suggestion alone. The mean Q-No score of these five participants was 13. These findings support the role of expectations in shaping both symptom improvement and worsening in clinical trials and clinical practice[39]. Prior studies likewise demonstrate that negative verbal suggestions can induce unpleasant symptoms or transform otherwise neutral sensations into painful experiences[40].

4.1 Argumentative of choosing to explore FC among DMN regions interictally in migraine during a “nocebo challenge”

Current evidence indicates widespread functional network alterations in migraineurs compared to healthy controls [41]. A recent meta-analysis highlighted the crucial role of the DMN in migraine pathogenesis, revealing abnormal FC in the right parahippocampal gyrus, MFG, and right PCC[42]. A dysfunctional neuro-limbic pain network may underlie the prominent role of stress and emotion as attack triggers, the prodromal and interictal migraine features, and the bidirectional influence of psychiatric comorbidity[43]. Stress has been shown to activate the DMN at rest[44], supporting the cortical hyperexcitability hypothesis in migraine[45]. Anxiety is also associated with placebo hyperalgesia[46].

Neuroimaging studies in healthy individuals on placebo hyperalgesia demonstrated activation in the anterior cingulate cortex (ACC), prefrontal cortex, insula, superior and middle temporal gyrus, and hippocampus during the pain anticipation[47–51]. Increased FC between hippocampus and pain network regions, including bilateral insula, operculum, ACC, and left primary somatosensory cortex/primary motor cortex as well as the periaqueductal gray matter (PAG), was demonstrated during placebo-induced hyperalgesia[52]. FC between the operculum and superior temporal gyrus (STG) has also been shown to increase over time, reflecting a mismatch between pain perception and expectation[53].

Based on this evidence, we aimed to investigate FC changes within DMN in response to a stress-induced placebo paradigm in individuals with migraine interictally.

4.2 Interpretation of the results of the FC differences among the experimental comparisons

4.2.1 1st Experimental Comparison

The absence of significant DMN connectivity differences between responders and non-responders, when all intervention groups were analyzed together, may suggest that placebo-related alterations may not emerge at a generalized level. Instead, such changes may depend on the specific experimental context, the nature of the placebo challenge, or may require larger sample sizes to be reliably detected.

4.2.2 2nd Experimental Comparison

To extricate experimentally induced placebo responses from general proneness, we compared DMN connectivity changes between clinically placebo-responsive participants and non-responders with $Q\text{-No} < 15$. This approach ensured that the observed alterations reflected the neural signature of an actual placebo response, rather than baseline questionnaire-defined susceptibility. Our results revealed decreased PCC and mPFC connectivity with left frontoparietal regions in responders, suggesting that placebo-related changes are specific and context-dependent, rather than a mere correlate of trait-level placebo proneness.

To interpret our findings, it is essential to consider the functions and interconnectivity of DMN regions. The PCC represents a central DMN hub[54, 55]. Ventral PCC via hippocampal connections, supports memory retrieval, future planning, and internally directed thought, while dorsal PCC demonstrates connections with frontal regions to modulate focus of attention, spatial processing, and expectancy-driven behavior[56, 57]. The MFG contributes to attention, working memory, decision-making, and shifting between internal and external focus[11, 58, 59]. The left inferior frontal gyrus (IFG) is engaged in semantic and phonological processing of speech, and empathy[58]. Notably, threat-related words activate the left retrosplenial PCC[60], suggesting that anticipatory processes may generate negative expectations and integrate them into sensory perception during pain processing[60]. Moreover, the prefrontal cortex (PFC) supports executive control, emotional regulation[61], and pain processing[62], with the mPFC playing a central role in fear memory[63], ventral portions in interoceptive regulation, and rostral and dorsal portions in affective appraisal[64]. Catastrophizing and pain vigilance have been associated with increased activity in the dorsolateral PFC, rostral ACC, and mPFC[65], highlighting the role of prefrontal circuits in expectancy-driven symptom amplification[66]. It is well known that anxious states, can increase the capacity to detect threats within the environment, by priming and focusing attentional and sensory systems[64, 67].

In our study, reduced connectivity between core DMN hubs (PCC, mPFC) and lateral frontoparietal regions suggests impaired integration of self-referential and attentional networks during the nocebo response. This disruption may indicate a shift toward internally generated negative expectations, consistent with the anticipation of adverse effects. Such a mechanism could underlie how verbal suggestions shape symptom perception in migraine. Additionally, right-sided numbness in nocebo responders coincided with reduced mPFC–left postcentral connectivity, suggesting expectancy-driven disruption of contralateral somatosensory processing, finding that further strengthens the view that expectancy-related mechanisms can directly shape bodily perception in migraine.

4.2.3 3rd Experimental Comparison

The frontal pole contributes to decision making, pain processing, working memory, and social cognition[68–70]. Through its connections with the ACC and orbitofrontal cortex, it participates in reward–punishment appraisal and attention switching[71]. It has been proposed that reward or punishment signals from these regions can destabilize the frontal pole, influencing other prefrontal networks to either exploit current options or explore alternatives[72]. Moreover, distinct ventromedial prefrontal regions have been shown to differentially respond to threat and safety, facilitating survival-related decisions[73]. Both the PCC and the frontal pole are central components of the DMN[11], interconnected through the cingulate fasciculus[74]. Prior studies have demonstrated heterogeneous changes in their activation, with some showing deactivation of the medial frontal pole alongside the PCC, lateral parietal, temporal, amygdala, and parahippocampal regions[75], while others reported enhanced activity during pain[76]. In chronic pain, FC within the DMN and between the DMN and the medial frontal pole is often disrupted, and this disruption has been linked to pain rumination, with higher rumination associated with stronger mPFC–PCC coupling[77]. Furthermore, during pain anticipation,

medial frontal pole and PCC deactivation has been observed, followed by reactivation during pain perception[78]. Interestingly, mindfulness meditation training has been associated with increased PCC–rostrolateral prefrontal connectivity, supporting improved attentional shifts to present-moment stimuli[79].

Consequently, the increased PCC–frontal pole connectivity observed in Experiment A responders may reflect enhanced integration of self-referential DMN activity with prefrontal regions involved in expectation and anticipatory processing, consistent with a neural signature of nocebo susceptibility.

4.2.4 4th Experimental Comparison

The postcentral gyrus encompasses the primary somatosensory cortex, while the lateral parietal cortex—including the superior parietal lobule (SPL), supramarginal gyrus, and angular gyrus—supports attention, working memory, spatial processing, and social cognition[80]. The SPL contributes to somatosensory and visuomotor integration[81] and visuospatial attention[82], whereas the inferior parietal lobule is implicated in volition[83], body image[84], and emotional spatial attention[85, 86]. The supramarginal gyrus further mediates the detection of unanticipated stimuli by reorienting attention to sensory input[87]. Given its central role in external attention, the parietal cortex is also thought to support similar internal processes, such as memory retrieval[88, 89].

In our study, the two nocebo responders in Experiment B not only showed reduced lateral parietal–postcentral connectivity but also had higher anxiety and depression scores compared to non-responders, suggesting that heightened trait anxiety may predispose individuals to attentional distraction by emotionally salient stimuli[90]. The reduced connectivity between the right lateral parietal cortex and the right postcentral gyrus in Experiment B responders may indicate disrupted integration of attentional and somatosensory processes. This disruption is consistent with the reported numbness, suggesting that negative verbal suggestion can bias sensory perception through altered parietal–somatosensory coupling[91].

4.3 Limitations and Strengths of the study

The present study has certain limitations. The major limitation is the small sample size, with only five clinical nocebo responders, limiting statistical power and generalizability, though the inclusion of migraine patients—a population highly susceptible to nocebo effects—may have facilitated detection of subtle FC changes. Unequal randomization and differences in years of education across groups could have influenced subgroup comparisons, although education was included as a covariate. The absence of a healthy control group precluded direct comparison of migraine-specific versus general nocebo alterations; however, our within-subject pre/post design reduced inter-individual variability. While Q- No questionnaire intended to capture trait-like predispositions, its administration between scanning sessions may also be influenced by state-dependent factors. A further limitation is that only numbness was assessed as a nocebo-induced symptom and was evaluated through self-report. Therefore, the findings may not be generalizable to other manifestations of the nocebo phenomenon and may not fully capture its complexity. Future studies should investigate a broader range of nocebo-related symptoms

using complementary objective measures. Also, the exclusive focus on DMN connectivity may overlook the contribution of other neural systems implicated in placebo responses; future work should consider examining multiple networks to provide a more comprehensive account of the underlying mechanisms. Finally, the lack of a placebo arm prevented direct comparison of placebo and placebo mechanisms. A strength of our design was restricting comparisons to responders and non-responders with low Q-No scores, which increased specificity but may reduce generalizability to individuals with high trait-level placebo susceptibility.

5. CONCLUSIONS

Our findings provide novel evidence of altered functional connectivity within the DMN in placebo-prone individuals with migraine during the interictal phase, in the context of an anxiety-induced placebo challenge. These alterations may reflect reduced integration between self-referential and attentional networks. Future studies with larger, more balanced samples, including healthy control groups, are warranted to validate and extend these findings and to identify reliable neuroimaging correlates of placebo susceptibility in migraine.

Abbreviations

dACC= dorsal anterior cingulate cortex, PFC= prefrontal cortex, STG= superior temporal gyrus, SPL= superior parietal lobule, PAG= periaqueductal gray matter, rs- fMRI= resting- state functional Magnetic Resonance Imaging, FC= functional connectivity, DMN= default mode network, ICHD-3= International Classification of Headache Disorder III edition, TR= repetition time, TE=time echo, FLAIR= fluid attenuated inversion recovery, HAM-A= Hamilton Anxiety Rating Scale, HAM-D= Hamilton Depression Rating Scale, SCL-90-R= Symptom Checklist-90-Revised scale, HR= High Resolution, BOLD= blood-oxygen-level-dependent, MNI= Montreal Neurological Institute, SPM12= Statistical Parametric Mapping 12, mPFC= medial prefrontal cortex, PCC= posterior cingulate cortex, S.D.= standard deviation, ANOVA= analysis of variance, ANCOVA= analysis of covariance, FWE= family-wise error, HSD=honestly significant difference, MFG= middle frontal gyrus, SMG= supramarginal gyrus, IFG= inferior frontal gyrus, IPL= inferior parietal lobule

Declarations

Declaration of interests: None

Ethics approval and consent to participate

All procedures were approved by the Aeginition Hospital Institutional Review Board (approval number 905/05-12-2018) and complied with the ethical standards of the Declaration of Helsinki.

Consent for publication

All participants provided written informed consent prior to participation.

Availability of data and materials

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Authors' contributions

Conception and design of the study: D.D. Mitsikostas, E. Kararizou, I. Spanou

Acquisition, analysis, and interpretation of data: I. Spanou, E. Karavasilis, F. Christidi, G. Velonakis

Drafting a significant portion of the manuscript or figures: I. Spanou, E. Karavasilis, F. Christidi

Manuscript editing: I. Spanou, D.D. Mitsikostas

Acknowledgements

We are indebted to all patients who generously gave their time to participate in this study.

Supplementary material

Basic demographic and clinical features of 36 participants are included in the Appendix.

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Figures

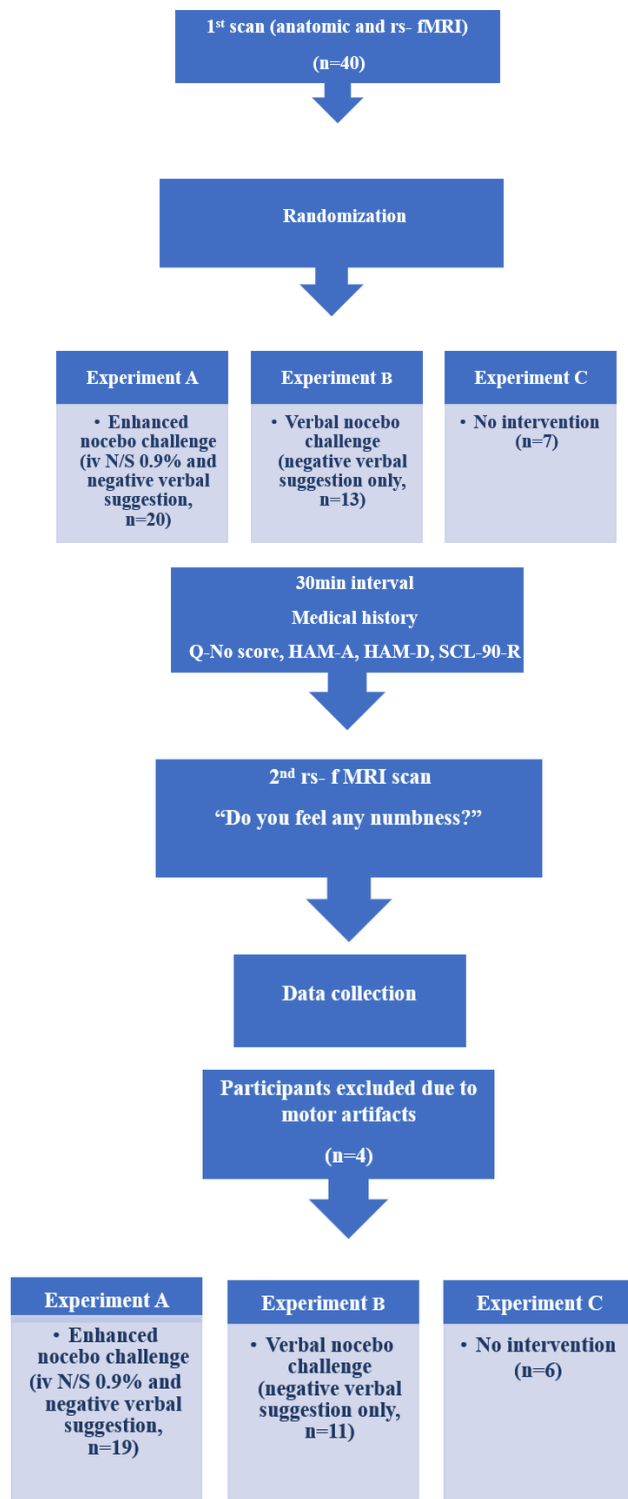


Figure 1

The experimental flow chart

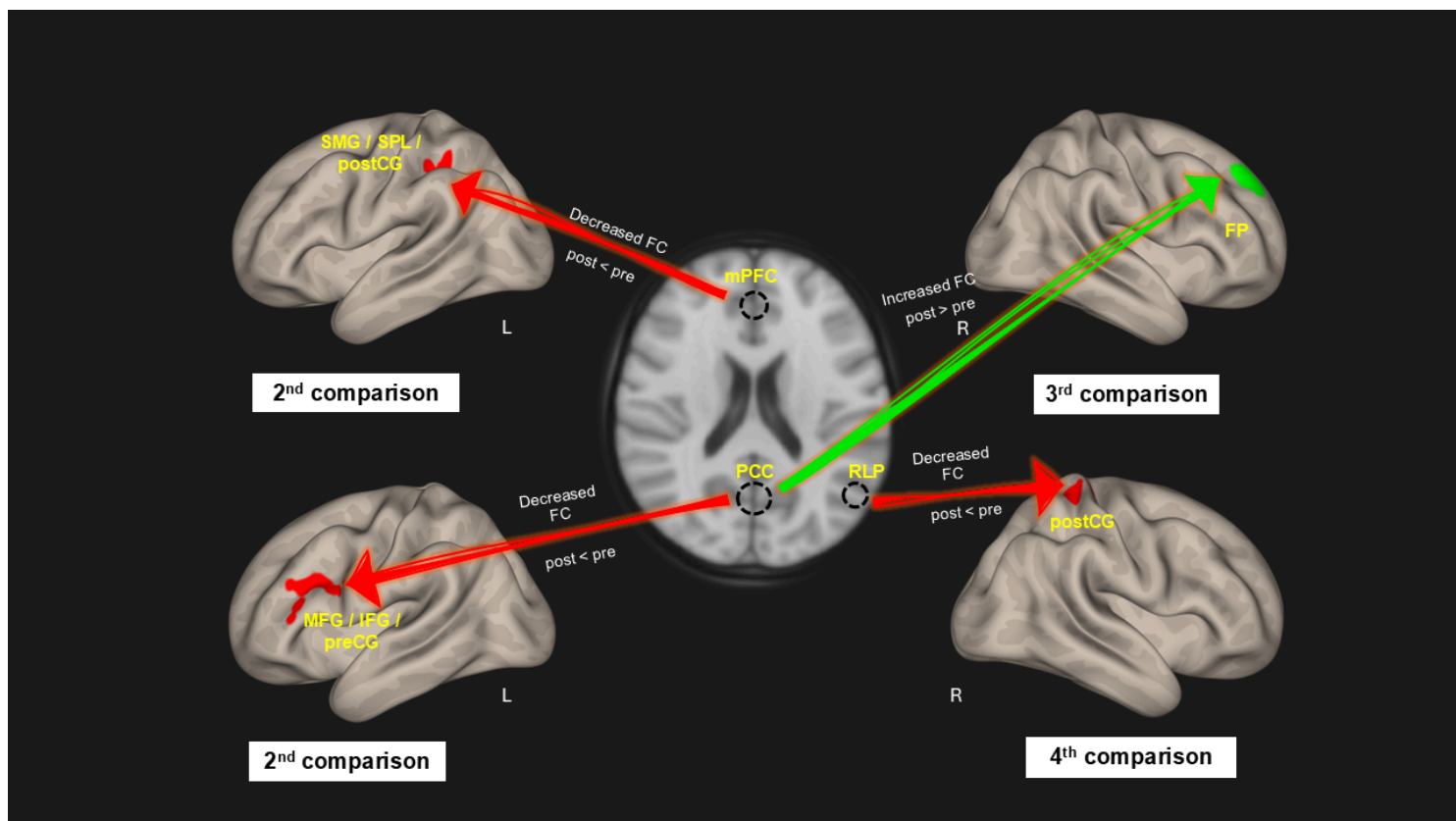


Figure 2

Functional Connectivity among Default Mode network regions through the different study's experimental comparisons

Abbreviations in Figure 2: FC= functional connectivity, SMG= supramarginal gyrus, SPL= superior parietal lobule, post-CG= post central gyrus, MFG= middle frontal gyrus, IFG= inferior frontal gyrus, pre-CG=precentral gyrus, FP= frontal pole, mPFC= medial prefrontal cortex, PCC= posterior cingulate cortex, RLP= right lateral parietal, R=right, L=left

Supplementary Files

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- [AppendixDemographicandclinicalfeaturesofparticipants.docx](#)