Neurophysiologie Clinique 52 (2022) 58–68

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

ORIGINAL ARTICLE

EEG-heart rate connectivity changes after sensorimotor rhythm neurofeedback training: Ancillary study

Guzmán Alba[a], Juan L. Terrasa[b], Jaime Vila[a], Pedro Montoya[b], Miguel A. Muñoz[a,*]

[a] *Brain, Mind and Behavior Research Center at University of Granada (CIMCYC-UGR), Spain*
[b] *Research Institute of Health Sciences (IUNICS), University of Balearic Islands, Palma, Spain*

Received 25 May 2021; accepted 23 November 2021
Available online 12 December 2021

KEYWORDS
EEG;
Heart rate;
Neurofeedback;
Sensorimotor rhythm;
Functional connectivity;
Fibromyalgia

Abstract
Objectives: Neurofeedback can induce long-term changes in brain functional connectivity, but its influence on the connectivity between different physiological systems is unknown. The present paper is an ancillary study of a previous paper that confirmed the effect of neurofeedback on brain connectivity associated with chronic pain. We analysed the influence of neurofeedback on the connectivity between the electroencephalograph (EEG) and heart rate (HR).
Methods: Seventeen patients diagnosed with fibromyalgia were divided into three groups: good sensorimotor rhythm (SMR) training responders (n = 4), bad SMR responders (n = 5) and fake training (SHAM, n = 8). Training consisted of six sessions in which participants learned to synchronize and desynchronize SMR power. Before the first training (pre-resting state) and sixth training (post-resting state) session, open-eye resting-state EEG and electrocardiograph signals were recorded.
Results: Good responders reduced pain ratings after SMR neurofeedback training. This improvement in fibromyalgia symptoms was associated with a reduction of the connectivity between the central area and HR, between central and frontal areas, within the central area itself, and between central and occipital areas. The sham group and poor responders experienced no changes in their fibromyalgia symptoms.
Conclusions: Our results provide new evidence that neurofeedback is a promising tool that can be used to treat of chronic pain syndromes and to obtain a better understanding of the interactions between physiological networks. These findings are preliminary, but they may pave the

* Corresponding author at: Brain, Mind and Behavior Research Center at University of Granada (CIMCYC-UGR), Campus de la Cartuja SN, C.P. 18071, Granada, Spain.
E-mail address: mamuoz@ugr.es (M.A. Muñoz).

https://doi.org/10.1016/j.neucli.2021.11.003

way for future studies that are more methodologically robust. In addition, new research questions are raised: what is the role of the central-peripheral network in chronic pain and what is the effect of neurofeedback on this network.

Introduction

The brain is a complex network of dynamic connections between local and distant brain areas [72]. Interregional neural communication is thought to be accompanied by a synchronization of oscillations between different brain regions [22]. This synchronization can be analysed by means of functional connectivity, which is a measure of the statistical dependency between activity in different brain regions. In recent years, investigations in the field of functional connectivity have been extended from relations between brain regions, to integration with peripheral systems [5,30]. These investigations have included an analysis of the interactions between different physiological signals (for example, electromyography, electrocardiography (EKG), electroencephalography (EEG), electrodermal activity, etc.) as a biomarker of neuropathological and mental states [5,27,41,61]. The underlying rationale is that the human body consists of an integrated network, where multi-component systems continuously interact through various feedback mechanisms to optimize the function of organs [5]. For example, it has been demonstrated that brain functional connectivity is related to cardiovascular homeostasis at rest [1,10,15,58].

Neurofeedback (NFB) is a non-invasive technique whose objective is that individuals learn to regulate certain parameters of cortical activity such as amplitude, frequency and/or coherence of EEG signal [28]. The main learning mechanism underlying NFB is operant conditioning [20]. During NFB, individuals learn to modify their cortical activity through the visual or acoustic information (feedback) that acts as a reinforcement in training [20,68]. Many studies have shown both structural [24] and functional [56] changes in brain networks after NFB training [2,49,50,55]. The influence of NFB training is not limited to neural activations related to a single trained channel, but changes the functional connectivity involving other brain areas, extending throughout the brain [70]. For example, it has been found that NFB training in sensorimotor rhythm (SMR, 12—15 Hz) in C3 and C4 decreases functional connectivity between frontal and temporal electrodes [11]. Moreover, the influence of NFB training extends beyond the brain and can affect peripheral systems. Studies have found that SMR NFB training increased heart rate variability (HRV) and decreased heart rate (HR) in healthy participants [4,52,53]. Thus, modifying brain activity through NFB can cause changes in heart rate, reflecting the synchronization of the two systems. However, these studies were conducted under the assumption that the brain and cardiac systems are correlated, but they were not considered as two interconnected nodes that belong to the same network. If we consider that the physiology of the human body is a complex interconnected network, NFB should lead to changes in the functional connectivity between remote nodes.

It has been observed that chronic pain causes profound changes to the brain and peripheral systems [3,57]. For example, patients with chronic pain exhibit a sympathetic predominance which explains a higher heart rate, [39,44,54,71] and an increase in EEG connectivity between central and frontal areas, [16,25] compared to healthy controls. NFB is a potential non-pharmacological treatment for the management of chronic pain conditions [8,31,33,34,43,73]. Modulating SMR is a procedure commonly used in NFB, which has been found to impact well-being in people with chronic pain [32,33]. The rationale is to reduce the connectivity and over-activation of lower beta rhythm (12—15 Hz) that patients with chronic pain exhibit in the resting-state over motor, somatosensory and frontal areas [25,26,40]. Similarly, feedback research has shown that increasing heart rate variability is related to enhancement of the SMR and has a positive effect on psychological health in patients with fibromyalgia [40, 41]. Nevertheless, the effect of SMR NFB training on heart rate remains unexplored in patients with fibromyalgia. As previous studies have found that SMR NFB training is related to changes in HR activity [23,24], so SMR NFB training should decrease functional connectivity between somatosensory and motor-related areas and HR activity [16,25].

This is an ancillary study to a previous paper (Terrasa et al. [67]), which aims to provide new data about the benefits of NFB in the treatment of chronic pain and the changes in EEG-HR connectivity associated with the improvement seen after training in patients with chronic pain. If we consider that the physiology of the human body comprises a complex interconnected network, then acting on one of these individual systems should lead to changes in the dynamics of other physiological systems and reorganize the characteristics of the physiologic network. We chose to investigate functional connectivity between somatosensory areas and heart rate due to studies that show the bidirectional relation between SMR NFB and HR. Moreover, SMR NFB has been used successfully in the treatment of chronic pain. We hypothesize that SMR NFB training decreases functional connectivity of electrodes located in somatosensory and motor-related areas with other brain areas and HR activity.

Methods

Participants

Seventeen right-handed female patients (aged 54.94 ± 10.11 years) with a diagnosis of fibromyalgia were recruited from the Asociación Granadina de Fibromialgia (AGRAFIM) in Granada (Spain). The diagnosis of fibromyalgia was confirmed following the American Rheumatology College Criteria [76]. The exclusion criteria were having been diagnosed with fibromyalgia for less than 1 year, being pregnant, exhibiting vision or auditory deficits, and having neurological or psychiatric diseases (except depression). Thirteen of the

seventeen patients had a diagnosis of depression. Participants continued to take regular medications, including analgesic/myorelaxant (88.24%), antidepressant (76.47%), and anxiolytic (70.59%) medications during the experiment, but were asked to avoid the use of any other non-pharmacological therapy. The study was conducted in accordance with the Declaration of Helsinki (1991) and approved by the Ethics Committee of the Balearic Islands (Spain). Written informed consent was obtained from the participants after the experimental procedure was explained to them.

Procedure

The procedure was explained in detail in Terrasa et al. (2020) [67]. Patients were sequentially assigned to either SMR NFB training (SMR, n = 9) or a control group that received false feedback during the training (SHAM, n = 8). All participants completed several questions about demographics, and self-reported questionnaires: Beck Depression Inventory (BDI-II) [6], Fibromyalgia Impact Questionnaire (FIQ) [7] and Short-form Health Survey (SF-36) composed of 9 dimensions: "physical functioning", "role limitations: physical", "role limitations: emotional", "vitality", "mental health", "social functioning", "pain", "general health perception" and "change in health" [75]. The NFB training program consisted of 6 sessions divided into 3 sessions per week for 2 weeks. Before the first training session (Pre-resting state) and before the start of the sixth training session (Post-resting state), the State-Trait Anxiety Inventory (STAI-S) [64] questionnaire was completed and open-eye resting-state EEG and EKG signals were recorded for 7 min. At the end of first and sixth SMR NFB training sessions, patients were asked to rate their perceived pain using a numerical scale ranging from 0 to 100 (Pain Assessment Session).

Briefly, the goal of the EEG NFB task was to move a ball and hit a target localized on the right or left side of the computer screen using the Cursor Task module of the BCI2000 platform [60]. The participants endeavoured to synchronize (by increasing power amplitude) and to desynchronize (by decreasing power amplitude) the SMR at sensory-motor-related electrodes (C3, CP1, and CP5) to move the ball to the target.

Hitting the ball on the target acts as a positive reinforcement of learning to regulate SMR power [20]. Moreover, the number of trials in which the ball hit the target (percentage of hits) was recorded as the task performance index. The greater the change in SMR power effected by the participant, the greater the performance index achieved. In the first and sixth training sessions, all participants performed the NFB in an MRI scanner and received real feedback on their performance. The remaining four training sessions (second to fifth sessions) were performed in an MRI simulator and only the SMR group received real feedback on the changes in SMR power, while the SHAM group received random feedback.

Physiological data acquisition and pre-processing

In this paper, only EEG and EEG-HR functional connectivity results in pre-resting state and post-resting state are presented. The demographic data, psychological variables, EEG power acquired during NFB, fMRI functional connectivity and level of pain experienced were presented in the previous paper [67]. Physiological signals during the resting state were continuously acquired using Ag/AgCl electrodes with a 64-ch QuickAmp amplifier (Brain Products GmbH, Munich, Germany). EEG electrodes were mounted according to the 10/20 montage system, their impedance was maintained at $<$10 kOhm, and the sampling rate was 1000 Hz. The EKG signals were recorded with one electrode located on the left side of the back (near the heart). Both physiological signals were referenced online to the AFz electrode. EEG pre-processing analysis was performed with the EEGLAB toolbox for MATLAB [13]. The recordings were filtered offline with a 0.1-Hz high-pass filter and 70-Hz low-pass filter. All channels were offline-referenced to the average of the electrodes. EEG waveforms were segmented in epochs of 2 s in duration (a total of 210 epochs were obtained) for analyses. Bad epochs and channels were then eliminated using visual inspection and $\pm$70 mV amplitude difference in the epoch. Thus, nine of sixty-three EEG channels (FPz, AF7, AF8, FT9, FT10, F5, F6, TP7 and TP8) were removed due to faulty recording or because 20% of epochs were rejected. The longest recording had 210 epochs, and the shortest recording had 180 epochs. The mean number of epochs rejected was 12.76, and the standard deviation was 10.92. To equalize the number of epochs between participants, we selected the first 180 epochs after rejection in all recordings.

EKG signals were filtered offline with a 5-Hz high-pass filter and 45-Hz low-pass filter. R$-$R intervals were extracted from EKG data filtered using the ECGLAB toolbox for MATLAB [9]. Then, Kardia software [46] was used to obtain the weighted average of the HR every 2 s, obtaining a total of 210 HR values.

Functional connectivity in EEG activity between the central region and other cortical regions

Functional connectivity analysis was conducted with a self-programmed MATLAB script. First, the power spectral density of the EEG signals was estimated with Welch's averaged periodogram method using a Hamming window that has a 500-point fast Fourier transformation and does not overlap. Then, coherence was calculated as the functional connectivity index in SMR. This index measures the linear correlation between two EEG signals, x(t) and y(t), as a function of the frequency, f. Thus, coherence is the ratio of the cross-power spectral density, Sxy(f), between both signals and their individual power spectral densities, Sxx(f) and Syy(f):

$$K_{xy}(f) = \frac{S_{xy}(f)}{\sqrt{S_{xx}(f)S_{yy}(f)}}$$

To reject spurious correlations between cortical sources, the imaginary part of coherence (iCOH) was calculated in each EEG epoch between central electrodes (Fc5, Fc3, Fc1, Fc2, Fc4, Fc6, C5, C3, C1, Cz, C2, C4, C6, Cp5, Cp3, Cp1, Cpz, Cp2, Cp4, and Cp6) and the rest of the EEG electrodes. The mean iCOH over time was obtained for each link of the central EEG network. We decided to use both right- and left-hemisphere electrodes in the central areas because of the low spatial resolution of the EEG signals and the fact that the use of NFB training over C3, CP1 and CP5 electrodes

shows contralateral desynchronization and ipsilateral synchronization over central areas.

Functional connectivity between EEG activity and HR

This analysis was conducted with a self-programmed MATLAB script. The SMR power was calculated in each EEG epoch as the sum of power spectral densities between 12 and 15 Hz in central electrodes. The length of the HR time series was set to be equal to the SMR power time series to eliminate the epochs rejected during EEG pre-processing. Both time series were z transformed to improve normality and stabilize variance. Finally, Pearson correlations were performed between the HR and SMR power of the central electrodes.

Statistical analysis

After the initial statistical analyses, we observed that participants in the SMR group as a whole could not achieve an average performance above chance level. Therefore, we decided to subdivide the SMR group into good responders (who achieved a mean performance level above 50% success during all the sessions) and poor responders (who achieved a mean performance level under 50% success during all the sessions). Thus, the study was finally conducted with three groups: good SMR responders (n = 4) with 67.76% $\pm$ 15.97 successful trials (mean of the six sessions), poor SMR responders (n = 5) with 48.31% $\pm$ 7.26 successful trials and sham group (n = 8). Task performance (percentage of success) for each group through the six sessions is shown in Fig. 1.

Statistical analyses were carried out using IBM SPSS Statistics v.23. For self-reported questionnaires (SF-36, BDI-II, and FIQ) one-way analyses of variance (ANOVAs) were used to examine differences between groups (good-SMR responders, bad-SMR responders and SHAM). Differences in STAI-S scores were tested using 3 $\times$ 2 repeated-measures ANOVA with group as the between-subject factor and resting-state session (Pre-resting state and Post-resting state) as the within-subject factor. Similarly, differences in pain perception at the end of first and sixth SMR NFB training sessions were tested using 3 $\times$ 2 repeated-measures ANOVA with group as the between-subject factor and Pain Assessment Session (First assessment and Last-assessment) as the within-subject factor. Finally, functional connectivity measures (ICOH, correlations EEG-HR) consisted of 3 $\times$ 2 repeated-measures ANOVA with group as the between-subject factor and resting-state session (Pre-resting state and Post-resting state) as the within-subject factor.

For repeated-measures analyses, normal distributions of the measured variables were tested, and Greenhouse−Geisser epsilon corrections were applied to control for violation of the sphericity assumption. When significant effects were found, post hoc analyses were performed using Bonferroni correction.

Results

Demographic and psychological data

Before SMR NFB training, there were no significant differences between groups with respect to depression (BDI-II) scores, but there was a significant difference in FIQ scores [$F(2, 14) = 4.16$, $p < .05$, $\eta_\rho^2 = 0.41$]. Bonferroni post hoc analyses of FIQ only showed a non-significant difference between good SMR and poor SMR responders on these scores ($p = .062$). SF-36 questionnaire analysis showed significant differences between groups in SF36-pain [$F(2, 15) = 4.12$, $p < .05$, $\eta_\rho^2 = 0.39$], SF36-general health perception [$F(2, 15) = 5.95$, $p < .05$, $\eta_\rho^2 = 0.48$] and SF36-change in health [$F(2, 15) = 7.13$, $p < .01$, $\eta_\rho^2 = 0.52$] before SMR NFB training. Bonferroni post hoc analyses of these effects revealed that good-SMR responders had higher scores than poor SMR responders on SF36-pain ($p < .05$; 39.7 $\pm$ 21.5 and 9.2 $\pm$ 12.6, respectively), SF36-general health perception ($p < .05$, 41.2 $\pm$ 14.3 and 12.0 $\pm$ 12.5, respectively) and SF36-change in health ($p < .01$, 43.7 $\pm$ 12.5 and 5.0 $\pm$ 12.1, respectively).

The ANOVA 3 $\times$ 2 analysis showed no significant differences on STAI-S scores between groups, resting-state

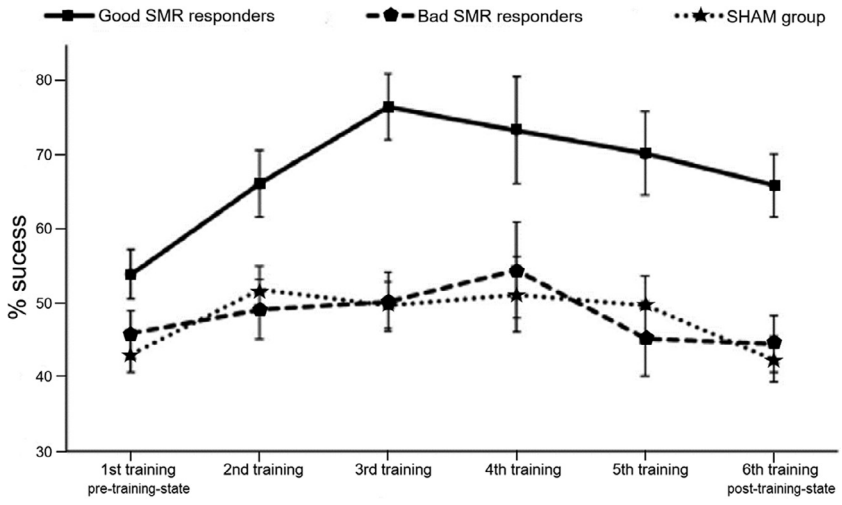

Fig. 1 Percentage of success in each group across training sessions.

Table 1 Significant F-test results and partial eta values of 3 × 2 repeated-measures ANOVAs for each network physiology node.

Fp1Cz	F(2,14)=9.61**	η_ρ^2=0.58	Fp1Fc1	F(2,14)=4.52*	η_ρ^2=0.39
Fp1Fc2	F(2,14)=7.87**	η_ρ^2=0.53	Fp1Fc3	F(2,14)=4.12*	η_ρ^2=0.37
Fp1C5	F(2,14)=7.69**	η_ρ^2=0.52	Fp2C5	F(2,14)=8.18**	η_ρ^2=0.54
F3C4	F(2,14)=4.12*	η_ρ^2=0.37	F3Cz	F(2,14)=5.29*	η_ρ^2=0.43
F3Fc1	F(2,14)=4.83*	η_ρ^2=0.41	F3Fc2	F(2,14)=5.15*	η_ρ^2=0.42
F3Cp2	F(2,14)=4.95*	η_ρ^2=0.41	F3C1	F(2,14)=5.56*	η_ρ^2=0.44
F3C2	F(2,14)=4.11*	η_ρ^2=0.37	F4C5	F(2,14)=6.16*	η_ρ^2=0.47
C3Fz	F(2,14)=4.07*	η_ρ^2=0.37	C3Fc2	F(2,14)=3.75*	η_ρ^2=0.35
C4O1	F(2,14)=4.57*	η_ρ^2=0.40	C4Fz	F(2,14)=6.30*	η_ρ^2=0.47
C4Fc1	F(2,14)=6.82**	η_ρ^2=0.49	C4Cp5	F(2,14)=4.99*	η_ρ^2=0.42
C4F1	F(2,14)=4.95*	η_ρ^2=0.41	C4Fc3	F(2,14)=6.21*	η_ρ^2=0.47
C4Cp3	F(2,14)=5.11*	η_ρ^2=0.42	P4Cz	F(2,14)=4.16*	η_ρ^2=0.37
P4Fc1	F(2,14)=5.75*	η_ρ^2=0.45	P4Fc2	F(2,14)=7.29**	η_ρ^2=0.51
O2Fc1	F(2,14)=3.96*	η_ρ^2=0.36	O2Fc3	F(2,14)=5.55*	η_ρ^2=0.44
F7Fc1	F(2,14)=4.10*	η_ρ^2=0.37	F7Fc2	F(2,14)=3.80*	η_ρ^2=0.35
F8Fc1	F(2,14)=5.24*	η_ρ^2=0.43	P7Fc2	F(2,14)=3.89*	η_ρ^2=0.36
P8Fc3	F(2,14)=4.23*	η_ρ^2=0.38	FzCz	F(2,14)=4.12*	η_ρ^2=0.37
FzFc1	F(2,14)=7.72**	η_ρ^2=0.53	FzFc2	F(2,14)=4.78*	η_ρ^2=0.41
FzCp2	F(2,14)=6.67**	η_ρ^2=0.49	FzCp6	F(2,14)=4.13*	η_ρ^2=0.37
FzC1	F(2,14)=6.07*	η_ρ^2=0.46	FzC2	F(2,14)=4.64*	η_ρ^2=0.40
FzCp4	F(2,14)=4.21*	η_ρ^2=0.38	FzC5	F(2,14)=8.74**	η_ρ^2=0.56
CzOz	F(2,14)=4.18*	η_ρ^2=0.37	CzPoz	F(2,14)=4.98*	η_ρ^2=0.42
CzF1	F(2,14)=3.90*	η_ρ^2=0.36	CzAF4	F(2,14)=7.34**	η_ρ^2=0.51
CzPo7	F(2,14)=3.87*	η_ρ^2=0.36	PzC5	F(2,14)=5.02*	η_ρ^2=0.42
OzFc1	F(2,14)=4.91*	η_ρ^2=0.41	OzFc2	F(2,14)=4.87*	η_ρ^2=0.41
Fc1Fc2	F(2,14)=5.02*	η_ρ^2=0.42	Fc1Cp2	F(2,14)=4.32*	η_ρ^2=0.38
Fc1Poz	F(2,14)=7.37**	η_ρ^2=0.51	Fc1AF3	F(2,14)=4.01*	η_ρ^2=0.36
Fc1AF4	F(2,14)=4.60*	η_ρ^2=0.40	Fc2C1	F(2,14)=4.01*	η_ρ^2=0.36
Fc2AF3	F(2,14)=5.13*	η_ρ^2=0.42	Fc2Cp4	F(2,14)=5.07*	η_ρ^2=0.42
Fc2Po8	F(2,14)=4.47*	η_ρ^2=0.39	Cp2Po3	F(2,14)=3.78*	η_ρ^2=0.35
Fc5F2	F(2,14)=3.80*	η_ρ^2=0.35	Fc5AF3	F(2,14)=4.82*	η_ρ^2=0.41
Fc5HR	F(2,14)=4.10*	η_ρ^2=0.37	Cp5AF3	F(2,14)=8.10**	η_ρ^2=0.54
Cp5Cp4	F(2,14)=4.09*	η_ρ^2=0.37	Cp5Po4	F(2,14)=4.20*	η_ρ^2=0.38
Cp6Po3	F(2,14)=4.59*	η_ρ^2=0.40	Cp6Po4	F(2,14)=4.40*	η_ρ^2=0.39
Cp6Ft7	F(2,14)=3.75*	η_ρ^2=0.35	Cp6HR	F(2,14)=4.50*	η_ρ^2=0.39
F1Fc3	F(2,14)=6.11*	η_ρ^2=0.47	F1C5	F(2,14)=5.69*	η_ρ^2=0.45
F1Cpz	F(2,14)=4.74*	η_ρ^2=0.40	F2C5	F(2,14)=3.94*	η_ρ^2=0.36
C1AF3	F(2,14)=5.96*	η_ρ^2=0.46	C2AF3	F(2,14)=4.54*	η_ρ^2=0.39
P1C5	F(2,14)=4.91*	η_ρ^2=0.41	AF3Fc3	F(2,14)=6.24*	η_ρ^2=0.47
AF4Fc3	F(2,14)=3.86*	η_ρ^2=0.36	AF4C5	F(2,14)=5.10*	η_ρ^2=0.42
Fc3Po8	F(2,14)=4.79*	η_ρ^2=0.41	Fc4HR	F(2,14)=4.13*	η_ρ^2=0.37
Cp3C5	F(2,14)=5.08*	η_ρ^2=0.42	Cp4Po3	F(2,14)=6.78**	η_ρ^2=0.49
Po3C6	F(2,14)=9**	η_ρ^2=0.56	C6HR	F(2,14)=4.73*	η_ρ^2=0.40

session or interaction between factors (all $p < .05$). No significant differences in pain perception were found by ANOVA 3 × 2 analysis between groups or pain assessment sessions. However, analysis yielded a significant interaction effect of group x pain assessment session [F(2, 14) = 4.103, $p < .05$, η_ρ^2 = 0.37]. The Bonferroni post hoc tests showed that good SMR responders reported lower levels of pain than poor SMR responders ($p < .05$) in the last pain assessment session. No significant group differences were found in level of pain experienced after the first training session. Good SMR responders also reported a significant reduction in the level of pain experienced between first and sixth training session ($p < .05$), which was not seen in r either poor-SMR responders or sham participants.

Functional connectivity in eeg activity between the central region and other cortical regions

A 3 × 2 repeated-measures ANOVA on iCOH was performed for each link between the central electrodes and the rest of the EEG electrodes. For clarity, only significant results related to group by session are reported, while the main effects of resting-state session or group are not reported. Table 1 displays the *F*s and partial etas of group x resting-state session interactions between EEG and HR connectivity.

Bonferroni *post hoc* tests between groups revealed that good SMR responders presented higher iCOH in 5 links (Fc2Fp1, Fc4P4, Fc2F7, Fc1Fz and Fc2Fz) than SHAM responders in the pre-resting-state session. In the post-resting-state session, good SMR responders had lower iCOH in the

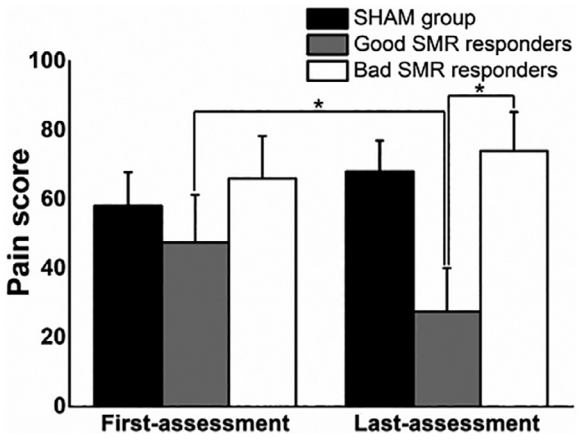

Fig. 2 Pain ratings during the Pain Assessment Session (first assessment and last-assessment) for each group ($p < .05$).

Fc1F8 link than poor SMR responders. Poor SMR responders presented higher iCOH in 8 links (CzFp1, C1F3, Fc1F8, C1Fz, CzF1, CzAF4, CpzF1 and C1AF3) and lower iCOH in 2 links (Fc5F2 and Fc5AF3) than sham responders in the post-resting-state session.

Post hoc analysis of the interaction comparing pre- and post-resting-state sessions in each group yielded significant differences in 42 links in good SMR responders, 30 links in poor SMR responders and only 11 links in the sham group (Fig. 2). In summary, good SMR responders had decreased iCOH in central-frontal, central-central, central-parietal and central-occipital links in post-resting-state sessions. Poor SMR responders presented a random pattern of increases and decreases between central, frontal and occipital links in the post-resting-state session. Finally, the sham group mainly had increased iCOH on some central-frontal, central-central, central-parietal and central-occipital links in the post-resting-state session. Thus, NFB training changed resting EEG functional connectivity more than the SHAM training condition, and good performance in SMR modulation was related to a decrease in iCOH after training.

Functional connectivity between eeg activity and HR

The functional connectivity between SMR power in central electrodes and heart rate reached significant group x resting-state session interactions in the Fc5, Fc4, C6 and Cp6 electrodes (Table 1). Bonferroni *post hoc* tests showed that good SMR responders decreased their connectivity in Fc5HR, Fc4HR and C6HR links in post-resting state-sessions (Fig. 3). However, poor SMR responders increased their connectivity in Cp6HR in the post-resting-state session. The sham group showed no significant differences in functional connectivity between central electrodes and heart rate.

Fig. 4

Bonferroni *post hoc* tests between groups showed that good SMR responders had higher SMR power-HR connectivity of SMR power on Fc5HR and Cp6HR than poor SMR responders in the pre-resting-state session. Moreover, good SMR responders showed higher SMR power-HR connectivity in the Fc5HR group than in the sham group. No significant differences between groups in the pre-resting-state session in Fc4HR and C6HR or differences between groups in the post-resting-state session were found.

Discussion

The aim of this study was to provide novel data about the benefits of NFB in the treatment of chronic pain pathologies and the changes in EEG-HR connectivity associated with the improvement seen in patients with chronic pain after the training. A cohort of patients with fibromyalgia was randomly assigned to an SMR training group or to a sham group. The analyses of task performance during the six sessions revealed that 4 out of 9 participants in the SMR training group were able to achieve a success rate above 50%. Good SMR responders reduced their perceived pain after SMR NFB training, while the SHAM group and bad SMR responders did not reduce their pain level. Good SMR responders showed reduced connectivity between central and frontal areas, within the central area itself, between central area and occipital area and between the central area and heart rate activity after SMR NFB training. However, the sham group showed increases in EEG functional connectivity, while the poor SMR responder group showed both decreases and increases in EEG functional connectivity. In relation to connectivity between central SMR activity and heart rate activity, good SMR responders decreased their connectivity in Fc5HR, Fc4HR and C6HR links after NFB training, while poor SMR responders increased functional connectivity in Cp6HR links after NFB training. These results support the view that NFB training induced changes in functional connectivity between cortical regions and between brain and peripheral autonomic activities, and that these changes are associated with improvements in patients with chronic pain. Specifically, it was observed that SMR NFB training reduced functional connectivity between EEG activity in the central cortical region (situated over motor and sensory areas), and heart rate. Moreover, functional connectivity changes remained stable during the resting-state evaluation.

Good SMR responders learned to successfully synchronize and desynchronize the SMR through the sessions, which was not achieved by the sham group and poor responders. Success in the SMR NFB training appears to be associated with relief of pain, because only good SMR responders decreased their perceived pain after the training. Previous research has linked SMR NFB training with reductions in anxiety [23], although this was not shown in our study.

Thus, the neural correlate of good SMR responders was a decrease in functional connectivity of central regions with frontal, parietal, and occipital regions and with nodes within the central region itself pertaining to the SMR when comparing pre- and post-resting-state sessions. On the other hand, poor SMR responders showed an unclear pattern of increases and decreases in EEG functional connectivity of central activity with frontal and occipital nodes and within the central region itself, while the sham group showed increased functional connectivity of the central region with frontal, parietal and occipital nodes, although these changes only affected eleven links. It is well known that patients with chronic pain show overactivation in frequency ranges between 12 and 15 Hz over central areas in the resting state [36,59,65,74], as well as strengthened connectivity of

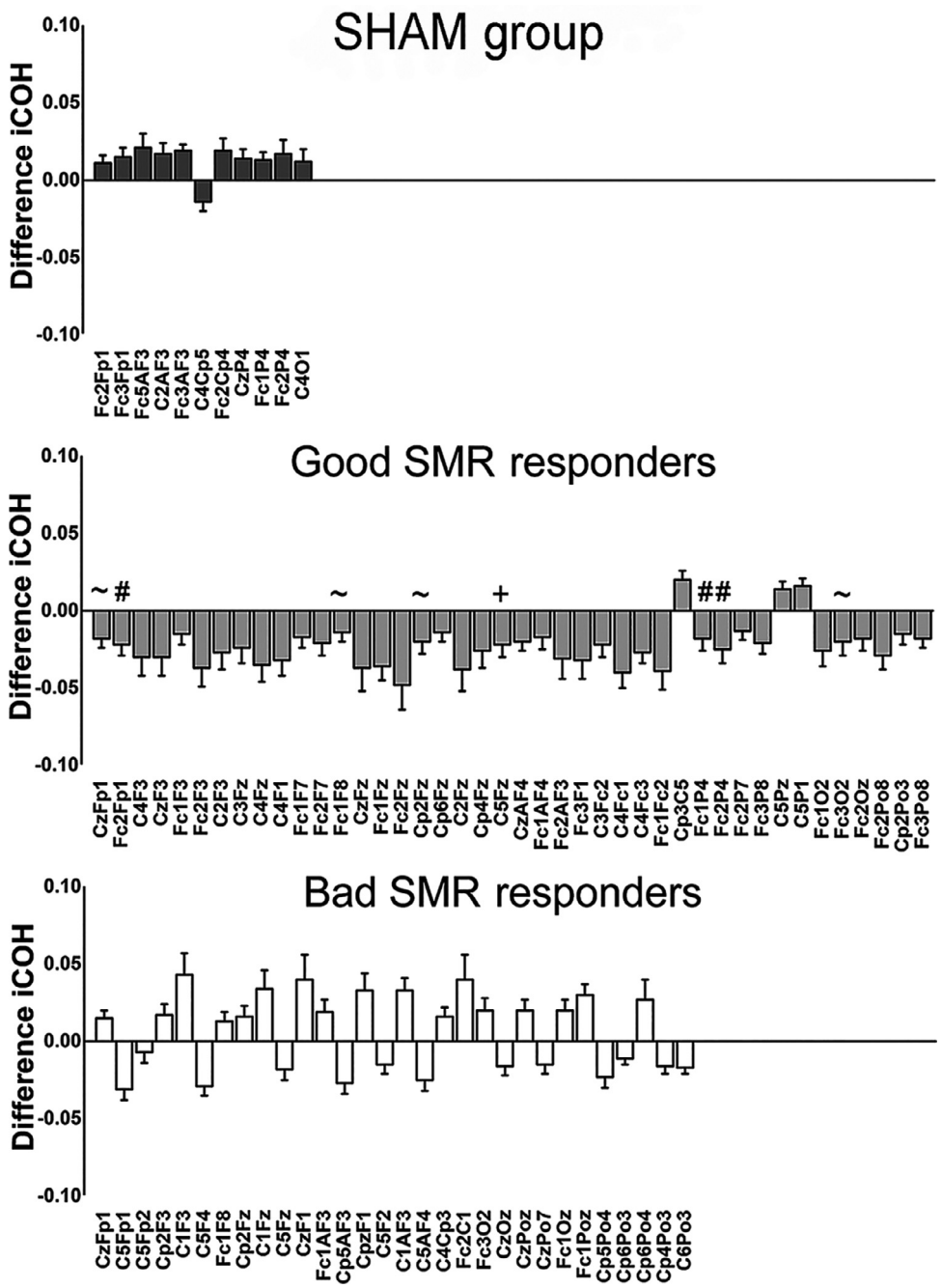

Fig. 3 Means of differences and standard errors of significant differences ($p < .05$) in central EEG links between post- and pre-SMR resting-state sessions. The symbols indicate if increases and decreases in common links between groups were equal or different (# good $\neq$ SHAM; $\sim$ good $\neq$ bad; + good = bad).

somatosensory regions with brain regions involved in pain processing [12,21,29]. Our EEG results are consistent with previous fMRI results, as SMR NFB altered the EEG connectivity in electrodes closely localised to somatosensory and motor-related areas in the resting state. These results are supported by previous studies showing that SMR NFB training reduces the coherence of central electrodes with other EEG areas [37,51]. Moreover, our results seem to indicate that a decrease in the connectivity of central areas with other brain regions could be related to the effectiveness of NFB training in providing pain relief [8,34,38,45]. SMR NFB training and relief of pain could be related to the learning of an EEG pattern characterized by reduced connectivity between central areas and areas related to pain processing, while an EEG pattern characterized by random connectivity could reflect difficulties in training in NFB. To our knowledge, there are no previous studies testing how NFB training could modulate the connectivity between central and peripheral systems, considering heart rate as another node. It is well known that HR has an effect on EEG activity [63,69]. For example, Prinsloo et al. (2011) [48] observed that HRV biofeedback training induces higher theta/beta

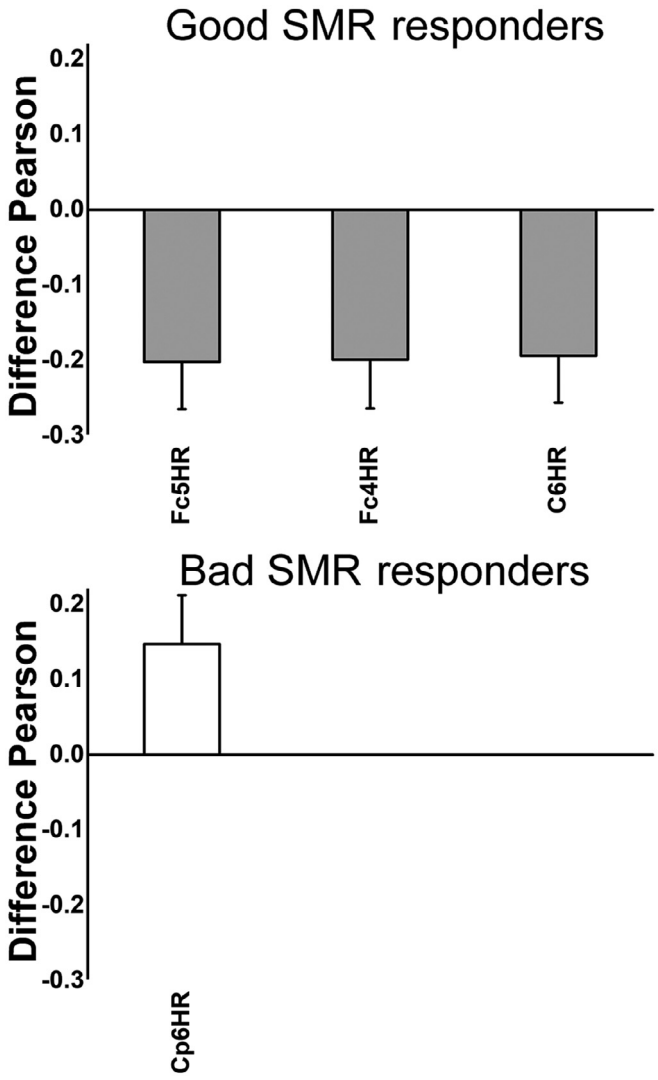

Fig. 4 Means and standard errors of significant differences ($p < .05$) in connectivity between EEG activity and heart rate between post- and pre-SMR sessions.

ratios in the frontal, central and parietal areas. Similarly, HRV NFB training increases SMR levels in central localizations, and SMR NFB training produces a decrease in HR [4,52]. Thompson and Thompson (2009) [70] support the existence of neural synergy between physiological systems, which proposes that NFB performed at Cz will affect not only activity in the central region but also affects whole physiological networks (in both central and autonomic nervous systems). This study is the first to show that NFB in central areas could induce long-term changes in functional connectivity related to an improvement in chronic pain symptoms, not only regarding EEG activity but also regarding heart rate. Some studies indicate that brain areas related to pain processing and neuronal networks involved in cardiovascular regulation are closely integrated [17—19,42,62]. Moreover, a recent study demonstrated that altered primary somatosensory cortex connectivity of patients with fibromyalgia is related to heart rate variability [35]. Studies of the somatic aspects of patients with chronic pain patients have shown that the autonomic state of patients with fibromyalgia is characterized by increased sympathetic and decreased parasympathetic tone in the resting state [44,54,71] with consequent increased heart rate than in heathy volunteers.

Previous studies showed that NFB can modify heart rate in healthy volunteers [4,52]. We expected similar results in patients with chronic pain, who indeed showed changes in the functional connectivity between central brain regions and HR, at least diminished in good SMR responders. Nevertheless, the connectivity changes in Fc5HR and Cp6HR links should be considered with caution because these links showed differences between groups in the pre-resting-state session. The differences in baseline EEG-HR connectivity are difficult to explain. A tentative explanation could be related with a higher pain impact over frontal and central cortical regions, which could be preferentially involved in NFB learning [16,25]. In general, our findings suggest that physiological networks could be shaped by experience-driven modulation of SMR NFB, and training could cause long-term changes in the interactions between physiological systems. Moreover, our results also suggest that patients with fibromyalgia show altered functional connectivity between the somatosensory cortex and the heart. This means that central

and autonomic physiological alterations, which occur in chronic pain disorders, might influence each other.

The design of the current study entails some shortcomings; therefore, its findings should be interpreted with caution. First and most importantly, the sample size was small (the statistical power was 50.98% considering f = 0.25, α error = 0.05 and correlation of repeated measures = 0.4), which makes the findings only preliminary. Secondly, the fact that all participants took regular medication during NFB training could have biased the results; hence, the possible effects of medication on the connectivity changes observed in this study should be further explored. Finally, the quality of the reinforcement used in the NFB training was not controlled and this could reduce the rate of successful self-regulation of SMR power. Previous studies have shown that the use of a motivationally relevant reinforcement can increase the effectiveness of NFB training [14,47,66]. Future research controlling the quality of the reinforcement could increase the proportion of participants who learn to self-regulate SMR power by NFB successfully.

Our results are an extension of previous results published [67] providing new evidence that NFB training is a promising tool that can be used in the treatment of chronic pain syndromes and to obtain a better understanding of the interactions between physiological networks. Beyond the limitations of the study, these results may pave the way for future methodologically more robust studies showing the capacity of neurofeedback to modulate the connectivity between the central and peripheral systems. Moreover, our results indicate that the analysis of the central-peripheral network could help in the treatment of patients with chronic pain and to investigate its physiological basis. This work opens two new research lines: the effect of NFB on the connectivity between different physiological systems and the relationship of physiological networks with chronic pain symptoms.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any professional, commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This work was supported by the Spanish Ministry of Economy and Competitiveness (PSI2014—57231-R).

References

[1] Alba G, Vila J, Rey B, Montoya P, Muñoz MA. The relationship between heart rate variability and electroencephalography functional connectivity variability is associated with cognitive flexibility. Front Hum Neurosci 2019;13:1–12.

[2] Amano K, Shibata K, Kawato M, Sasaki Y, Watanabe T. Learning to associate orientation with color in early visual areas by associative decoded fMRI neurofeedback. Curr Biol 2016;26:1861–6.

[3] Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. Pain 2011;152:S49.

[4] Balt K, Du Toit P, Smith M, van Rensburg C. The the effect of infraslow frequency neurofeedback on autonomic nervous system function in adults with anxiety and related diseases. NeuroRegulation 2020;7:64–74.

[5] Bartsch RP, Liu KKL, Bashan A, Ivanov PC. Network Physiology: how Organ Systems Dynamically Interact. PLoS ONE 2015;10: e0142143.

[6] Beck AT, Steer RA, Ball R, Ranieri WF. Comparison of beck depression inventories-IA and-II in psychiatric outpatients. J Pers Assess 2010;67:588–97.

[7] Burckhardt CS, Clark SR, Bennett RM. The fibromyalgia impact questionnaire: development and validation. J Rheumatol 1991;18:728–33.

[8] Caro XJ, Winter EF. EEG biofeedback treatment improves certain attention and somatic symptoms in fibromyalgia: a pilot study. Appl Psychophysiol Biofeedback 2011;36:193–200.

[9] de Carvalho JLA, da Rocha AF, de Oliveira Nascimento FA, Neto JS, Junqueira LF. Development of a Matlab software for analysis of heart rate variability. 6th Int Conf Signal Process, 2002. IEEE 2002;2:1488–91.

[10] Chand T, Li M, Jamalabadi H, Wagner G, Lord A, Alizadeh S, et al. Heart rate variability as an index of differential brain dynamics at rest and after acute stress induction. Front Neurosci 2020;14.

[11] Cheng MY, Wang KP, Hung CL, Tu YL, Huang CJ, Koester D, et al. Higher power of sensorimotor rhythm is associated with better performance in skilled air-pistol shooters. Psychol Sport Exerc 2017;32:47–53.

[12] Cifre I, Sitges C, Fraiman D, Muñoz MÁ, Balenzuela P, González-Roldán A, et al. Disrupted functional connectivity of the pain network in fibromyalgia. Psychosom Med 2012;74:55–62.

[13] Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004;134:9–21.

[14] Diaz Hernandez L, Rieger K, Koenig T. Low motivational incongruence predicts successful EEG resting-state neurofeedback performance in healthy adults. Neuroscience 2018;378:146–54.

[15] Ding K, Tarumi T, Wang C, Vernino S, Zhang R, Zhu DC. Central autonomic network functional connectivity: correlation with baroreflex function and cardiovascular variability in older adults. Brain Struct Funct 2020;225:1575–85.

[16] Dinh ST, Nickel MM, Tiemann L, May ES, Heitmann H, Hohn VD, et al. Brain dysfunction in chronic pain patients assessed by resting-state electroencephalography. Pain 2019;160:2751.

[17] Edwards L, Inui K, Ring C, Wang X, Kakigi R. Pain-related evoked potentials are modulated across the cardiac cycle. Pain 2008;137:488–94.

[18] Edwards L, McIntyre D, Carroll D, Ring C, Martin U. The human nociceptive flexion reflex threshold is higher during systole than diastole. Psychophysiology 2002;39:678–81.

[19] Edwards L, Ring C, McIntyre D, Carroll D. Modulation of the human nociceptive flexion reflex across the cardiac cycle. Psychophysiology 2001;38:712–8.

[20] Enriquez-Geppert S, Huster RJ, Herrmann CS. EEG-neurofeedback as a tool to modulate cognition and behavior: a review tutorial. Front Hum Neurosci 2017;11:1–19.

[21] Flodin P, Martinsen S, Löfgren M, Bileviciute-Ljungar I, Kosek E, Fransson P. Fibromyalgia is associated with decreased connectivity between pain- and sensorimotor brain areas. Brain Connect 2014;4:587–94.

[22] Fries P. A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn Sci 2005;9:474–80.

[23] Gadea M, Aliño M, Hidalgo V, Espert R, Salvador A. Effects of a single session of SMR neurofeedback training on anxiety and cortisol levels. Neurophysiol Clin 2020;50:167–73.
[24] Ghaziri J, Tucholka A, Larue V, Blanchette-Sylvestre M, Reyburn G, Gilbert G, et al. Neurofeedback training induces changes in white and gray matter. Clin EEG Neurosci 2013;44:265–72.
[25] González-Roldán AM, Cifre I, Sitges C, Montoya P. Altered dynamic of EEG oscillations in fibromyalgia patients at rest. Pain Med 2016;17:1058–68.
[26] González-Villar AJ, Triñanes Y, Gómez-Perretta C, Carrillo-de-la-Peña MT. Patients with fibromyalgia show increased beta connectivity across distant networks and microstates alterations in resting-state electroencephalogram. Neuroimage 2020;223:117266.
[27] Gould van Praag CD, Garfinkel SN, Sparasci O, Mees A, Philippides AO, Ware M, et al. Mind-wandering and alterations to default mode network connectivity when listening to naturalistic versus artificial sounds. Sci Rep 2017;7:1–12.
[28] Gruzelier JH. EEG-neurofeedback for optimising performance. I: a review of cognitive and affective outcome in healthy participants. Neurosci Biobehav Rev 2014;44:124–41.
[29] Ichesco E, Schmidt-Wilcke T, Bhavsar R, Clauw DJ, Peltier SJ, Kim J, et al. Altered resting state connectivity of the insular cortex in individuals with fibromyalgia. J Pain 2014;15:815–26.
[30] Ivanov PCH, Liu KKL, Bartsch RP. Focus on the emerging new fields of network physiology and network medicine. New J Phys 2016;18:100201.
[31] Jensen MP, Day MA, Miró J. Neuromodulatory treatments for chronic pain: efficacy and mechanisms. Nat Rev Neurol 2014;10:167–78.
[32] Jensen MP, Gertz KJ, Kupper AE, Braden AL, Howe JD, Hakimian S, et al. Steps toward developing an eeg biofeedback treatment for chronic pain. Appl Psychophysiol Biofeedback 2013;38:101–8.
[33] Jensen MP, Hakimian S, Sherlin LH, Fregni F. New insights into neuromodulatory approaches for the treatment of pain. J Pain 2008;9:193–9.
[34] Kayıran S, Dursun E, Dursun N, Ermutlu N, Karamürsel S. Neurofeedback intervention in fibromyalgia syndrome; a randomized, controlled, rater blind clinical trial. Appl Psychophysiol Biofeedback 2010;35:293–302.
[35] Kim J, Loggia ML, Cahalan CM, Harris RE, Beissner F, Garcia RG, et al. The somatosensory link in fibromyalgia: functional connectivity of the primary somatosensory cortex is altered by sustained pain and is associated with clinical/autonomic dysfunction. Arthritis Rheumatol 2015;67:1395–405.
[36] Kim JA, Bosma RL, Hemington KS, Rogachov A, Osborne NR, Cheng JC, et al. Neuropathic pain and pain interference are linked to alpha-band slowing and reduced beta-band magnetoencephalography activity within the dynamic pain connectome in patients with multiple sclerosis. Pain 2019;160:187–97.
[37] Kober SE, Witte M, Stangl M, Väljamäe A, Neuper C, Wood G. Shutting down sensorimotor interference unblocks the networks for stimulus processing: an SMR neurofeedback training study. Clin Neurophysiol 2015;126:82–95.
[38] Kravitz HM, Esty ML, Katz RS, Fawcett J. Treatment of fibromyalgia syndrome using low-intensity neurofeedback with the flexyx neurotherapy system: a randomized controlled clinical trial. J Neurother 2006;10:41–58.
[39] Kulshreshtha P, Deepak KK, Yadav RK, Mukherjee D. Cardiac autonomic neuropathy in fibromyalgia: revisited. J Back Musculoskelet Rehabil 2021;Preprint:1–7.
[40] Lim M, Kim JS, Kim DJ, Chung CK. Increased low- and high-frequency oscillatory activity in the prefrontal cortex of fibromyalgia patients. Front Hum Neurosci 2016;0:111.
[41] Liu KKL, Bartsch RP, Lin A, Mantegna RN, Ivanov PC. Plasticity of brain wave network interactions and evolution across physiologic states. Front Neural Circuits 2015;9:1–15.
[42] Martins AQ, Ring C, McIntyre D, Edwards L, Martin U. Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. Pain 2009;147:84–90.
[43] Mayaud L, Wu H, Barthélemy Q, Favennec P, Delpierre Y, Congedo M, et al. Alpha-phase synchrony EEG training for multi-resistant chronic low back pain patients: an open-label pilot study. Eur Spine J 2019;28:2487–501.
[44] Meeus M, Goubert D, De Backer F, Struyf F, Hermans L, Coppieters I, et al. Heart rate variability in patients with fibromyalgia and patients with chronic fatigue syndrome: a systematic review. Semin Arthritis Rheum 2013;43:279–87.
[45] Mueller HH, Donaldson CCS, Nelson DV, Layman M. Treatment of fibromyalgia incorporating EEG-Driven stimulation: a clinical outcomes study. J Clin Psychol 2001;57:933–52.
[46] Perakakis P, Joffily M, Taylor M, Guerra P, Vila J. KARDIA: a Matlab software for the analysis of cardiac interbeat intervals. Comput Methods Programs Biomed 2010;98:83–9.
[47] Pérez-Elvira R, Oltra-Cucarella J, Carrobles JA, Moltó J, Flórez M, Parra S, et al. Enhancing the effects of neurofeedback training: the motivational value of the reinforcers. Brain Sci 2021;11:457.
[48] Prinsloo GE, Rauch HGL, Lambert MI, Muench F, Noakes TD, Derman WE. The effect of short duration heart rate variability (HRV) biofeedback on cognitive performance during laboratory induced cognitive stress. Appl Cogn Psychol 2011;25:792–801.
[49] Ramot M, Kimmich S, Gonzalez-Castillo J, Roopchansingh V, Popal H, White E, et al. Direct modulation of aberrant brain network connectivity through real-time NeuroFeedback. Elife 2017;6:1–23.
[50] Rance M, Walsh C, Sukhodolsky DG, Pittman B, Qiu M, Kichuk SA, et al. Time course of clinical change following neurofeedback. Neuroimage 2018;181:807–13.
[51] Reichert JL, Kober SE, Neuper C, Wood G. Resting-state sensorimotor rhythm (SMR) power predicts the ability to up-regulate SMR in an EEG-instrumental conditioning paradigm. Clin Neurophysiol 2015;126:2068–77.
[52] Reid A, Nihon S, Thompson L, Thompson M. The effects of heart rate variability training on sensorimotor rhythm: a pilot study. J Neurother 2013;17:43–8.
[53] Reneau M. Heart rate variability biofeedback to treat fibromyalgia: an integrative literature review. Pain Manag Nurs 2020;21:225–32.
[54] Reyes del Paso GA, Garrido S, Pulgar Á, Martín-Vázquez M, Duschek S. Aberrances in autonomic cardiovascular regulation in fibromyalgia syndrome and their relevance for clinical pain reports. Psychosom Med 2010;72:462–70.
[55] Robineau F, Meskaldji DE, Koush Y, Rieger SW, Mermoud C, Morgenthaler S, et al. Maintenance of voluntary self-regulation learned through real-time fMRI neurofeedback. Front Hum Neurosci 2017;11:1–8.
[56] Ros T, Théberge J, Frewen PA, Kluetsch R, Densmore M, Calhoun VD, et al. Mind over chatter: plastic up-regulation of the fMRI salience network directly after EEG neurofeedback. Neuroimage 2013;65:324–35.
[57] Rosselló F, Muñoz MA, Duschek S, Montoya P. Affective modulation of brain and autonomic responses in patients with fibromyalgia. Psychosom Med 2015;77:721–32.
[58] Sakaki M, Yoo HJ, Nga L, Lee TH, Thayer JF, Mather M. Heart rate variability is associated with amygdala functional connectivity with MPFC across younger and older adults. Neuroimage 2016;139:44–52.
[59] Sarnthein J, Stern J, Aufenberg C, Rousson V, Jeanmonod D. Increased EEG power and slowed dominant frequency in patients with neurogenic pain. Brain 2006;129:55–64.
[60] Schalk G, McFarland DJ, Hinterberger T, Birbaumer N, Wolpaw JR. BCI2000: a general-purpose brain-computer interface (BCI) system. IEEE Trans Biomed Eng 2004;51:1034–43.

[61] Schulz S, Haueisen J, Bär K-J, Voss A. Multivariate assessment of the central-cardiorespiratory network structure in neuropathological disease. Physiol Meas 2018;39:074004.
[62] Shao S, Shen K, Wilder-Smith EPV, Li X. Effect of pain perception on the heartbeat evoked potential. Clin Neurophysiol 2011;122:1838–45.
[63] Sherlin L, Muench F, Wyckoff S. Respiratory sinus arrhythmia feedback in a stressed population exposed to a brief stressor demonstrated by quantitative EEG and sLORETA. Appl Psychophysiol Biofeedback 2010;35:219–28.
[64] Spielberger C.D., Gorsuch R.L., Lushene R.E. The state-trait anxiety inventory (STAI) test manual for form X. 1970.
[65] Stern J, Jeanmonod D, Sarnthein J. Persistent EEG overactivation in the cortical pain matrix of neurogenic pain patients. Neuroimage 2006;31:721–31.
[66] Strehl U. What learning theories can teach us in designing neurofeedback treatments. Front Hum Neurosci 2014;0:894.
[67] Terrasa JL, Barros-Loscertales A, Montoya P, Muñoz MA. Self-Regulation of SMR power led to an enhancement of functional connectivity of somatomotor cortices in fibromyalgia patients. Front Neurosci 2020;14:1–14.
[68] Thibault RT, MacPherson A, Lifshitz M, Roth RR, Raz A. Neurofeedback with fMRI: a critical systematic review. Neuroimage 2018;172:786–807.
[69] Thompson M, Thompson L. Current practice of neurofeedback: where we are and how we got there. Biofeedback 2016;44:181–205.
[70] Thompson M, Thompson L. Systems theory of neural synergy: neuroanatomical underpinnings of effective intervention using neurofeedback plus biofeedback. J Neurother 2009;13:72–4.
[71] Tracy LM, Ioannou L, Baker KS, Gibson SJ, Georgiou-Karistianis N, Giummarra MJ. Meta-analytic evidence for decreased heart rate variability in chronic pain implicating parasympathetic nervous system dysregulation. Pain 2016;157:7–29.
[72] Varela F, Lachaux J-P, Rodriguez E, Martinerie J. The brainweb: phase synchronization and large-scale integration. Nat Rev Neurosci 2001;2:229–39.
[73] Vučković A, Altaleb MKH, Fraser M, McGeady C, Purcell M. EEG correlates of self-managed neurofeedback treatment of central neuropathic pain in chronic spinal cord injury. Front Neurosci 2019;13:1–17.
[74] Wang WE, Roy A, Misra G, Ho RLM, Ribeiro-Dasilva MC, Fillingim RB, et al. Altered neural oscillations within and between sensorimotor cortex and parietal cortex in chronic jaw pain. NeuroImage Clin 2019;24:101964.
[75] Ware JEJ, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–83.
[76] Wolfe F, Häuser W. Fibromyalgia diagnosis and diagnostic criteria. Ann Med 2011;43:495–502.