ORIGINAL ARTICLE

Assessing the Effectiveness of Neurofeedback for Drug-Resistant Focal Epilepsy MRI

Donya Abolghasemkhah-Salmasi [1, 2], Alireza Fallahi [2, 3], Narges Hoseini-Tabatabaei [4], Seyed-Sohrab Hashemi-Fesharaki [5], Reza Rostami [6], Mohammad-Reza Nazem-Zadeh [1, 2, 7*]

[1] Department of Medical Physics and Biomedical Engineering, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2] Research Center for Molecular and Cellular Imaging, Advanced Medical Technologies and Equipment Institute, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Biomedical Engineering, Hamedan University of Technology, Hamedan, Iran

[4] Medical School, Tehran University of Medical Sciences, Tehran, Iran

[5] Pars Advanced Medical Research Center, Pars Hospital, Tehran, Iran

[6] Department of Psychology, University of Tehran, Tehran, Iran

[7] Department of Neuroscience, Monash University, Melbourne, Australia

*Corresponding Author: Mohammad-Reza Nazem-Zadeh
Email: mnazemzadeh@tums.ac.ir

Received: 29 October 2023 / Accepted: 23 November 2023

Abstract

Purpose: Evidence shows that Neurofeedback (NF) can reduce seizure frequency and enhance Sensorimotor Rhythm (SMR) in patients with drug-resistant focal epilepsy, but the neural mechanisms underlying such effects are not well understood. The objective of this study was to investigate the neuromodulatory effects of SMR NF training on functional and structural connectivity in patients with drug-resistant focal epilepsy.

Materials and Methods: Four patients with drug-resistant focal epilepsy underwent functional Magnetic Resonance Imaging (fMRI), diffusion MRI (dMRI), quantitative electroencephalogram (QEEG), and Integrated Visual and Auditory (IVA-2) test before and after 6 to 8 weeks of SMR NF training. We assessed alterations in functional and structural connectivity within and between six brain networks based on the Automated Anatomical Labeling (AAL) atlas.

Results: All four patients showed a reduction of a minimum of 35% in seizure frequency after SMR NF training, with two patients experiencing a reduction within the first week of treatment. IVA-2 scores increased for all patients compared to the pre-treatment baseline, indicating cognitive improvement. Post-treatment fMRI revealed no significant differences in functional connectivity between patients and control cases, despite significant differences in some brain networks observed in pre-treatment fMRI. We also found increased fractional anisotropy (FA) values between subcortical and auditory networks after SMR training.

Conclusion: Our study provides promising evidence for the neural basis of SMR NF training in the treatment of drug-resistant focal epilepsy. The observed reductions in seizure frequency, improvements in cognitive abilities, and increased FA values suggest that SMR NF training may be an effective treatment for patients with drug-resistant focal epilepsy.

Keywords: Drug-Resistant Epilepsy; Neurofeedback; Sensorimotor Rhythm; Resting-State functional Magnetic Resonance Imaging; Diffusion Magnetic Resonance Imaging; Functional Connectivity; Structural Connectivity; Brain Networks.

DOI:

Frontiers in BIOMEDICAL TECHNOLOGIES

1. Introduction

Antiepileptic Drugs (AEDs) are the primary treatment for managing epilepsy [1]. However, more than 30% of patients with epilepsy continue to experience seizures despite routine pharmacological treatments [2], which is classified as Drug-Resistant Epilepsy (DRE). Clinical guidelines recommend against more than two consecutive pharmacological treatments and categorize such cases as refractory, suggesting invasive resective surgery if a primary surgical outcome can be anticipated without any postsurgical deficits [3]. Alternatively, over the past decade, research into nonpharmacological treatments with significantly fewer severe side effects has gained greater attention, particularly psychological management of epilepsy, including cognitive-behavioral therapy, biofeedback, and cognitive rehabilitation [4]. These treatments have shown promising effects in enhancing patients' cognitive states and improving their lifestyle [5-8].

Enhancement of the Sensorimotor Rhythm (SMR) through Neurofeedback (NF) has been found to be an effective treatment for patients who do not respond to AEDs [9-11]. Operant conditioning of the SMR (12-15 Hz) for the treatment of epilepsy has a 50-year history [12]. Neurophysiological studies have shown that operant learning of SMR training can reinforce the brain's ability to regulate the threshold of stimulation and prevent overstimulation and subsequent seizure activities [12].

Functional Magnetic Resonance Imaging (fMRI) can provide essential information assessing the neural bases and functional correlates of clinical improvements as treatment outcomes. Since its inception, this method has been widely employed in studies as a biomarker of clinical improvement in pharmacologic and training programs [13]. fMRI can be used to extract functional connectivity (FC) among brain regions based on Blood Oxygenation Level-Dependent (BOLD) signals [14]. FC can be defined as the temporal correlation among average time series of resting state fMRI (rs-fMRI) of different brain regions of interest [15].

Diffusion MRI (dMRI), on the other hand, is a quantitative MRI technique and one of the most common non-invasive methods for examining the structure of brain fiber tracts [16]. It quantifies the movement of water molecules [17], enabling the determination of the underlying microstructures that restrict or hinder free and isotropic movements. One of the valuable diffusion indices is Fractional Anisotropy (FA), which can evaluate the integrity of fiber tracts and specify the anatomical connectivity between functionally associated gray matter regions [18].

NF has been shown to improve cognitive and executive functions of the human brain, such as attention, which is a central component of cognitive ability [19]. Regarding the SMR training protocol, previous studies have reported reduced commission errors and improved perceptual sensitivity in a Continuous Performance Task (CPT), and general improvements in attention performance [20, 21]. The Integrated Visual and Auditory (IVA-2) test can be used to test two major attention control indices, visual attention and auditory attention. The IVA-2 attention and concentration test is a neuropsychological (neurocognitive), computer-based test (not a questionnaire) designed to evaluate attention in both visual and auditory domains, as well as checking impulse control performance. It examines the state of attention and concentration with high precision and determines the functioning of the nervous system related to attention and concentration [22].

Recent studies have examined the effectiveness of SMR training NF in epilepsy, using only a limited number of clinical measurements, such as seizure frequency, seizure severity, and electroencephalogram signals [9]. However, analyzing the main functional networks in the brain, including the Default-Mode Network (DMN), Sensorimotor Network (SMN), Visual Network (VIN), subcortical network (SCN), Auditory Network (AUN), and Attention Network (ATN), and their functional and structural intra- and interconnections can provide valuable information about the mechanism of NF efficacy on SMR training and how it can affect the brain's functional networks, eventually leading to more efficient treatment planning for epilepsy patients. Some of these brain networks have been reported to be different in epileptic patients compared to the healthy population [23, 24]. Therefore, we expected that post-NF neuroimaging data from patients with epilepsy would show fewer significant differences from the healthy cohort. We also aimed to find potential correlations between behavioral test results and neuroimaging findings after treatment to explain the NF effects on both patients' brains and behavior.

This study highlights the potential value of SMR training and provides valuable information on the effect of this non-invasive, non-pharmacological approach for

drug-resistant epilepsy cases. In this regard, multi-modal imaging, including fMRI and dMRI, was used in addition to conventional clinical and electrophysiological assessment modalities, to assess the clinical and functional improvements and their association in focal epilepsy patients.

2. Materials and Methods

2.1. Participants

We enrolled 4 patients with drug-resistant epilepsy between 18 and 35 years old in the treatment plan, randomly selected from the clients of Atieh Psychology Clinic in Tehran, if they met the inclusion and exclusion criteria (Table 1). The inclusion criteria were identifying persistent focal epilepsy (seizures originating from a limited number of spots within a hemisphere) based on previous EEG monitoring and seizure semiology confirmed by neurologists, with the experience of at least one seizure in the past 6 months. The participants were asked to maintain constant doses of their medication during the NF period and the next 3-month follow-up and to refrain from undergoing any interventional treatments, such as TMS, TDCS, etc., or surgery during this period. The exclusion criteria were: severe and recurrent generalized epilepsy, a history of brain surgery, or other neurological or cerebrovascular diseases previously treated with Vigabatrin (a drug with potential damage to visual processing). We also included 20 Healthy Controls (HCs) with no psychiatric or neurological history to measure the imaging-related variability through test-retest data acquisition and uncertainty analysis. All research participants voluntarily provided informed consent to enter the project. All patients' guardians received a seizure diary notebook and were asked to record the experienced seizures during the NF treatment and the 3-month follow-up. A questionnaire based on the Liverpool seizure severity scale was filled out at the start of the NF therapy, at the end of all NF sessions, and after the 3-month follow-up.

2.2. Attention Test

The IVA-2 test was taken to assess visual and auditory attention functions for each patient before and after the NF treatment. The IVA-2 is a 13-minute computerized test that combines two types of continuous performance tests for auditory and visual items. The task of this test is simply to ask the person to click on the computer mouse when hearing or seeing a specific target (number 1) and not to click on the mouse when something outside the target is presented (number 2). The questions are presented in a pseudo-random combination of visual and auditory stimuli, making it more demanding than other continuous performance tests in challenging a person's ability to change cognitive sets. In addition, the IVA-2 test can accurately differentiate five types of attention, including focused, continuous, selective, divided, and shifting attention in both visual and auditory levels. Like other continuous performance tests, the IVA-2 is designed as a repetitive and boring process that requires a high level of attention to measure errors caused by lack of attention and impulsivity during the process [22]. It reports the score of visual and auditory attention and where the person's scores stand in comparison with the normal population, which are normal, higher than normal, or lower than normal. In addition, the general indicators of attention (the score of general attention and the score of general impulsivity) quantify the individual's attention and concentration as well as impulsivity

Table 1. shows patient-related clinical and investigative features

No.	Sex	Age (Y)	Handedness	Epileptogenic Zone	Seizure Frequency			Medication	
					Pre NF	Post NF	3-month follow-up	Antiepileptic Drug 1	Antiepileptic Drug 2
1	Female	23	Right	Right Temporal	2-3/day	1/w	1/w	Carbamazepine 1200 mg/day	Clonazepam 2 mg/day
2	Female	25	Right	Left Parietal	1-2/w	-	-	Lamotrigine 25 mg/day	Zonisamide 400 mg/day
3	Female	33	Right	Right Temporal	3-4/w	1/w	1/w	Valproate sodium 800	Phenobarbital 200
4	Male	19	Right	Left Parietal	5-6/w	3-4/w	3/w	Valproate sodium 600	-

compared to healthy people of the same age and gender.

To review and analyze the data, Cohen's d method was used to calculate the effect size of the treatment and mean percentage improvement (MPI) based on the mean (M) and standard deviation (SD) of the obtained data. Ferguson (2009) reported values of 0.41, 1.15, and 2.70 for low, medium, and high effects, respectively, in single-subject designs, based on which we evaluated the data in our study [25] (Equations 1, 2).

$$MPI = \frac{average\ after\ treatment - average\ at\ baseline}{average\ after\ treatment} \times 100 \quad (1)$$

$$Cohen's\ d = \frac{M1 - M2}{S_{pooled}} \quad (2)$$

Where $S_{pooled} = \sqrt{(S_1^2 + S_2^2)/2}$

2.3. QEEG Acquisition

Pre- and post-EEG recordings were taken for QEEG analysis. The subjects' brain waves were recorded at rest and with eyes open and closed using 19-channel electrodes based on the international 10-20 system (Figure 1). Brain wave signals were analyzed and processed using Neuroguide software (version 3.0.5.0). Removal of artifacts (i.e. recorded waves of non-brain origin) was performed using visual inspection and computer selection methods. The absolute power and coherence scores of the electrodes were calculated for delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), and beta (12-25 Hz) frequencies. Using the Neuroguide normative database, all measured values were converted to standardized Z-scores.

2.4. Neurofeedback Protocol

Twenty sessions of one-hour NF treatments, three days a week, were conducted in the protocol, as the minimum requirement to measure the effect, as recommended in [27]. Before the start of the session, the participants were asked to sit on an armchair one meter away from the computer monitor in the therapy room. The seizure diary was checked, and the patient's guardians were asked if they had noticed any adverse reactions to the therapy. During the NF intervention, only the participant and the technician were present in

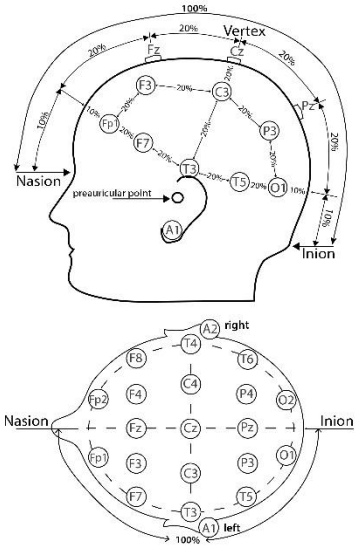


Figure 1. shows the placement of electrodes for EEG acquisition according to the international 10-20 system [26]

the room. Bio-Graph Infiniti software (version 5.1.3) was modified to standardize the functions for all patients. The active electrode was located at the midline central zone (CZ), and the reference electrodes were located at both earlobes. The pre-set parameters were as follows: inhibit-theta (4–7Hz) at least 20% below their threshold, reinforce-SMR (12–15Hz) 80% of the time, and inhibit-high-beta (25–35 Hz) at least 20% below their threshold. A game with three boats, each corresponding to a particular frequency band, was presented to the participants as a visual stimulus. Participants were asked to focus on the motion of the middle boat (the boat corresponding to the SMR frequency band). Whenever the participant reached the parameters for 0.5 seconds, the middle boat moved forward in the race while the other two boats stopped. The auditory stimulus was a bell. In addition, every time the participant completed the game path, they could see their points. Sample screens are shown in Figure 2. Participants received feedback on their performance at the end of each session and the technician recorded the high theta/SMR/beta threshold and total session scores.

2.5. MRI Data Acquisition

MRI data were acquired on a Siemens 3T Magnetom MRI scanner equipped with a 64-channel head coil at the Iranian National Brain Mapping Laboratory (NMBL). Rs-fMRI BOLD and dMRI data were acquired pre- and post-treatment for each patient.

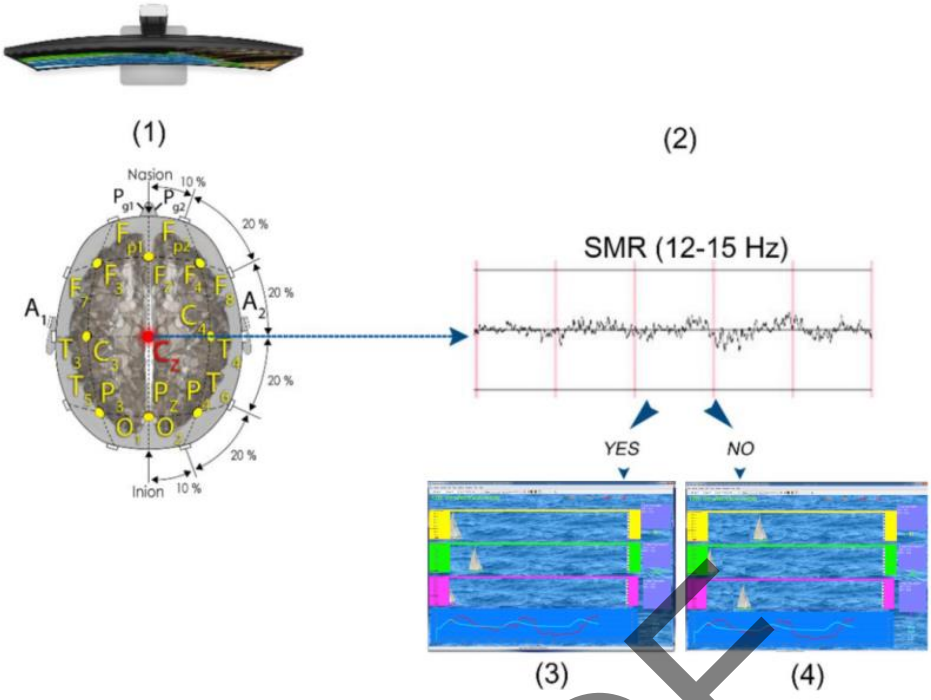


Figure 2. shows the SMR training neurofeedback protocol implemented in this study via BioGraph Infinity software. The active electrode was located at midline central zone (CZ), and the pre-set parameters were as follows: inhibit-theta (4–7Hz) at least 20% below their threshold, reinforce-SMR (12–15Hz) 80% of the time, and inhibit-high-beta (25–35 Hz) at least 20% below their threshold [28]

HCs underwent two separate test-retest imaging sessions on the same day, twenty minutes apart. The data acquisition parameters were exactly the same for the patient and HC groups.

The fMRI imaging parameters were as follows: T2-weighted Echo Planar Imaging (EPI) sequence (TR = 3000 ms, TE = 30 ms, FA = 90°, matrix = 640 × 640, 2.4 mm slice thickness, 53 slices, and 330 volumes). Participants were instructed to keep their eyes closed during the resting-state scans and to refrain from falling asleep or focusing on any specific thoughts. Foam padding was employed to minimize head movements. The diffusion imaging protocol consisted of 2 diffusion-weighted shells (1000, 2000 s/mm^2), 64 diffusion-weighted volumes each, and 5 reference volumes (b0 s/mm^2). For distortion correction, all images were additionally acquired with reversed-phase encoding. Other dMRI parameters were as follows: TR = 9600 ms, TE = 92 ms, flip angle = 90°, matrix = 880×880, 2.4 mm slice thickness, and 64 slices.

Subsequently, high-resolution T1 anatomical images were acquired for registration purposes using the 3D magnetization-prepared rapid gradient-echo sequence (MPRAGE) with the following parameters: TR = 1840 ms, TE = 2.43 ms, flip angle = 8°, matrix =256 × 256, 1.0 mm slice thickness, and 176 slices.

2.6. MRI Data Processing

2.6.1. Functional MRI

The DPABI (Data Processing & Analysis of Brain Imaging) toolbox version 4.3 [29] (http://rfmri.org/dpabi) was utilized to preprocess the rs-fMRI data. For each subject, the first 10 time points were discarded, and the remaining 320 volumes were corrected for the time difference between slices. They were then realigned to the middle volume using a six-parameter spatial transformation (rigid body). Skull stripping was performed to ensure proper registration of functional images to T1-weighted ones, and Head movement was corrected using motion scrubbing. The resulting images were segmented into Grey Matter (GM), White Matter (WM), and Cerebrospinal Fluid (CSF). The realigned functional volumes were spatially normalized to the Montreal Neurological Institute (MNI) space (https://nist.mni.mcgill.ca/) using the normalization parameters estimated from the

T1 structural images. To reduce the effect of low-frequency drifts, the dataset was subsequently smoothed and temporally filtered (0.01–0.08 Hz) using a Gaussian kernel (FWHM = 8 mm).

The time series of preprocessed rs-fMRI BOLD signals in voxels were averaged across each region of interest (ROI) located in brain cognitive networks based on the Automated Anatomical Labeling (AAL) atlas [30] (Table 2, Figure 3). The Fisher-transformed bivariate correlation coefficient was calculated between the average time series of each pair of ROIs. The resulting functional connectivity matrix (FCM) was extracted for each participant. Finally, the FCMs of the patient cohorts before and after SMR training were compared with the FCMs of the HC cohorts using a two-sample t-test with a significance level of $P < 0.05$. The t-test was used to determine whether there were significant differences in functional connectivity between the patient and HC cohorts. By examining the functional connectivity within and between these networks, we can gain insight into the neural mechanisms underlying the observed changes in behavior and cognition before and after SMR training.

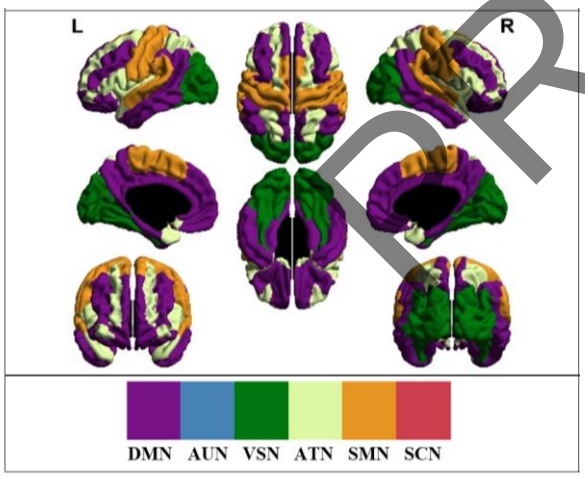


Figure 3. shows the brain functional networks based on AAL atlas, including the Default Mode Network (DMN), Attention Network (ATN), Sensorimotor Network (SMN), Visual Network (VSN), Subcortical Network (SCN), and Auditory Network (AUN)

2.6.2. Diffusion MRI

The DWI of patients was analyzed using ExploreDTI diffusion MRI software [31] (www.exploreDTI.com). Prior to data analysis and extracting the structural connectivity matrix (SCM) for each subject, the datasets were corrected for eddy current-induced geometric distortions and subject motion by realigning all diffusion-weighted volumes to the null b=0 volume. An affine transformation model and mutual information were used as the cost function [32]. After converting the data into NIFTI format, the B matrix was prepared using bvec and *.bvaluse files, which included the direction of the gradients and the amount of b-values taken. Using the B matrix and 4D NIFTI file, a DTI file was created and loaded into the DTI Explorer software. The DTI file was then visually inspected and processed in the pre-processing section [33].

Following this, the native file is the diffusion images on which corrections related to the patient's movement and magnetic field disturbances have been made, and the diffusion images registered on the T1 images are also created (trafo). The entire brain fibers were created from the trafo file using the tractography technique, based on the continuous tracking algorithm. Fiber tracking was stopped in voxels where Fractional Anisotropy (FA) < 0.2 or the angle between two eigenvectors of two consecutive voxels connected by tracking was greater than 30 degrees. Subsequently, each subject's tractogram was parcellated using 116 ROIs and 6 networks based on the AAL atlas. For acquiring mean FA, firstly, for each tract, the value of the underlying FA image is sampled at each vertex, and the mean of these values is calculated to produce a single scalar value of "mean FA" per fiber tract; as each tract is assigned to ROIs within the tractogram, the magnitude of the contribution of that tract to the matrix is multiplied by the mean FA value calculated prior for that tract.

The Fisher-transformed bivariate correlation coefficient was calculated between the mean FA of a pair of ROIs in the AAL atlas and the averaged mean FA values of the DMN, VSN, SCN, ATN, SMN, and AUN obtained from the patient's pre-processed DWI data. Then, the mean FA values in these networks were compared pre- vs. post-treatment for each subject using uncertainty assessment statistical analysis.

2.6.3. Uncertainty Assessment Statistical Analysis

To determine valid changes in the mean FA values of the networks (within/intra or between/inter

Table 2. provides a list of regions of interest (ROIs) that were included in each of the cognitive networks analyzed in the study. The cognitive networks include the default mode network (DMN), attention network (ATN), sensorimotor network (SMN), visual network (VSN), subcortical network (SCN), and auditory network (AUN)

Network	Regions	Abbreviation
DMN	Superior frontal gyrus, medial (Left) & (Right)	SFGmed.L, SFGmed.R
	Superior frontal gyrus, medial orbital (Left) & (Right)	ORBsupmed.L, ORBsupmed.R
	Anterior cingulate and paracingulate gyri (Left) & (Right)	ACG.L, ACG.R
	Median cingulate and paracingulate gyri (Left) & (Right)	MCG.L, MCG.R
	Posterior cingulate gyrus (Left) & (Right)	PCG.L, PCG.R
	Hippocampus (Left) & (Right)	HIP.L, HIP.R
	Parahippocampal gyrus (Left) & (Right)	PHG.L, PHG.R
	Inferior parietal, but supramarginal and angular gyri (Left) & (Right)	IPL.L, IPL.R
	Angular gyrus (Left) & (Right)	ANG.L, ANG.R
	Middle temporal gyrus (Left) & (Right)	MTG.L, MTG.R
	Temporal pole: middle temporal gyrus (Left) & (Right)	TPOmid.L, TPOmid.R
ATN	Superior frontal gyrus, dorsolateral (Left) & (Right)	SFGdor.L, SFGdor.R
	Superior frontal gyrus, orbital part (Left) & (Right)	ORBsup.L, ORBsup.R
	Middle frontal gyrus (Left) & (Right)	MFG.L, MFG.R
	Middle frontal gyrus orbital part (Left) & (Right)	ORBmid.L, ORBmid.R
	Inferior frontal gyrus, opercular part (Left) & (Right)	IFGoperc.L, IFGoperc.R
	Inferior frontal gyrus, triangular part (Left) & (Right)	IFGtriang.L, IFGtriang.R
	Inferior frontal gyrus, orbital part (Left) & (Right)	ORBinf.L, ORBinf.R
	Insula (Left) & (Right)	INS.L, INS.R
	Temporal pole: middle temporal gyrus (Left) & (Right)	TPOmid.L, TPOmid.R
	Inferior temporal gyrus (Left) & (Right)	ITG.L, ITG.R
SMN	Precentral gyrus (Left) & (Right)	PreCG.L, PreCG.R
	Supplementary motor area (Left) & (Right)	SMA.L, SMA.R
	Postcentral gyrus (Left) & (Right)	PoCG.L, PoCG.R
	Superior parietal gyrus (Left) & (Right)	SPG.L, SPG.R
	Supramarginal gyrus (Left) & (Right)	SMG.L, SMG.R
	Paracentral lobule (Left) & (Right)	PCL.L, PCL.R
VSN	Calcarine fissure and surrounding cortex (Left) & (Right)	CAL.L, CAL.R
	Precuneus (Left) & (Right)	PCUN.L, PCUN.R
	Lingual gyrus (Left) & (Right)	LING.L, LING.R
	Superior occipital gyrus (Left) & (Right)	SOG.L, SOG.R
	Middle occipital gyrus (Left) & (Right)	MOG.L, MOG.R
	Inferior occipital gyrus (Left) & (Right)	IOG.L, IOG.R
	Fusiform gyrus (Left) & (Right)	FFG.L, FFG.R
SCN	Caudate nucleus (Left) & (Right)	CAU.L, CAU.R
	Lenticular nucleus, putamen (Left) & (Right)	PUT.L, PUT.R
	Lenticular nucleus, pallidum (Left) & (Right)	PAL.L, PAL.R
	Thalamus (Left) & (Right)	THA.L, THA.R
AUN	Superior temporal gyrus (Left) & (Right)	STG.L, STG.R
	Temporal pole: superior temporal gyrus (Left) & (Right)	TPOsup.L, TPOsup.R

networks) for individual subjects, It is essential to estimate the corresponding Reproducibility Coefficient (RC) values as a measure of uncertainty. The RC is the uncertainty range estimated from test-retest data in longitudinal imaging studies.

To calculate the RC values, we used the one-way analysis of variance (ANOVA) model $Yik = \mu i + \varepsilon ik$, where Yik is the observed value for the i^{th} subject at the k^{th} replication ($i = 1, \dots n$; $k = 1, \dots K$; in our test and retest dataset, $n = 20, K = 2$). The observed values are expressed as the true value plus the within-subject error, with between-subject variance $\sigma 2B = Var(\mu i)$ and within-subject variance $\sigma 2W = Var(\varepsilon ik)$. The total variance is $\sigma 2T = \sigma 2B + \sigma 2W$.

$$WMS = \frac{1}{n(K-1)} \sum_{i=1}^{n} \sum_{k=1}^{K} (Yik - \bar{Y}i)^2 \qquad (3)$$

Where WMS is the within-means of squares, $\bar{Y}i$ is the mean of replications for i^{th}subject, and $\bar{Y}$ is the mean of all observations [34]. WMS can be estimated by $\widehat{\sigma_w^2}$ as:

$$\widehat{\sigma_w^2} = \frac{2}{n} \sum_{i=1}^{n} \left[\frac{Yit - Yir}{Yit + Yir}\right]^2 \qquad (4)$$

where t and r denote test and retest, respectively.

Using these estimates, we can determine the RC values and calculate the 95% Confidence Interval (CI) as follows [34] (Equations 5-7):

$$RC_L = 2.77\sqrt{\frac{n.WMS}{\chi^2_n(0.975)}} \quad (5)$$

$$RC_U = 2.77\sqrt{\frac{n.WMS}{\chi^2_n(0.025)}} \quad (6)$$

$$\widehat{RC}_\varepsilon\,(RC_L, RC_U) \quad (7)$$

By estimating the corresponding RC values, we can determine precisely how large an alteration in an imaging index (either longitudinal or interhemispheric in the brain) needs to be considered a real change. This approach allows us to compare an individual patient's change to the uncertainty range and determine whether it is statistically significant.

In the absence of disease progression or treatment, any change in a structure between test and retest is due to random or systematic errors originating from an imaging tool, such as MRI, image acquisition, patient repositioning, image processing and analysis, and/or subject physiological variations. Therefore, we can compare an individual patient's change to the estimated RC and determine whether it is statistically significant. If the change is outside the 95% CI, it is considered a true change, and we can attribute it to factors other than random or systematic errors.

3. Results

The patient-related clinical and investigative features are presented in Table 1. To compare the results of seizure frequency and seizure severity scale across the three time points in the study design (pre-, post-, and follow-up), a Friedman non-parametric test was utilized. The results of the test indicated that there were no significant differences in seizure severity scale ($\chi 2 = 2.8$, $p = 0.24$) as well as seizure frequency ($\chi 2 = 2.0$, $p = 0.36$). However, all participants reported a reduction in seizure frequency and severity after NF therapy, with a decrease ranging from 35-100% for seizure frequency and 5-15 % for seizure severity.

The study compared rs-fMRI data of focal epilepsy patients with HC (n = 20) data using functional connections between brain networks before and after SMR training NF. Before NF sessions, all patients showed some abnormal internetwork connections compared to HC. However, after NF, there was no alteration in FC between the patient and HC cohorts (Figure 4), suggesting that the cognitive network behavior in patients approached that of HCs after NF treatments. To assess the effect of SMR training on structural connectivity, the mean FA of SCM was compared pre and post-treatment using the uncertainty assessment statistical method, and communal alteration patterns in connections were examined. After treatment, the mean FA value between AUN and SCN increased in all patients (Figure 5).

Furthermore, there was a roughly 10% increase in average SMR power trend in patients after the NF therapy (Figure 6). All patients also showed considerable improvement in IVA-2 scores (Figure 7). Fig.8 illustrates the QEEG of focal epilepsy patients before and after NF therapy.

In order to determine the size of the therapy effect on the IVA-2 data, Cohen's d method was utilized. Table 3 presents the mean values, Standard Deviation (SD), Mean Percentage Improvement (MPI), and the effect size scores for each participant individually. The effect size values in three participants were greater than 0.41, which is considered a 'low to medium' effect according to the interpretation proposed by Ferguson [35]. For the second patient, the effect size was greater than 1.15, which is considered 'medium to high' according to the same scale. Patient 4 showed the highest MPI.

Patient 1

For patient 1, the visual and auditory attention scores were 79 and 59, respectively, before the NF therapy. After rehabilitation, the scores improved to 89 and 85, respectively, indicating an improvement in the patient's attention and response control (Figure 7).

Before the therapy, when Compared to the HC group (n=20), there was a 14.9% decrease in functional connectivity between AUN and ATN, and a 22% decrease between SMN and VSN in the patient's brain. However, after NF therapy, there was no significant difference in these connections compared to the HC group (Figure 4).

In this patient, there was an increase in FA values in the fiber trajectories between VIN-ATN, VIN-AUN, SCN-AUN, and SMN-AUN compared to pre-treatment (CI = 95%) (Figure 5).

Patient 2

For patient 2, the visual and auditory attention scores increased from 105 and 107 (before NF) to 109 and 110 (after NF), respectively, indicating an

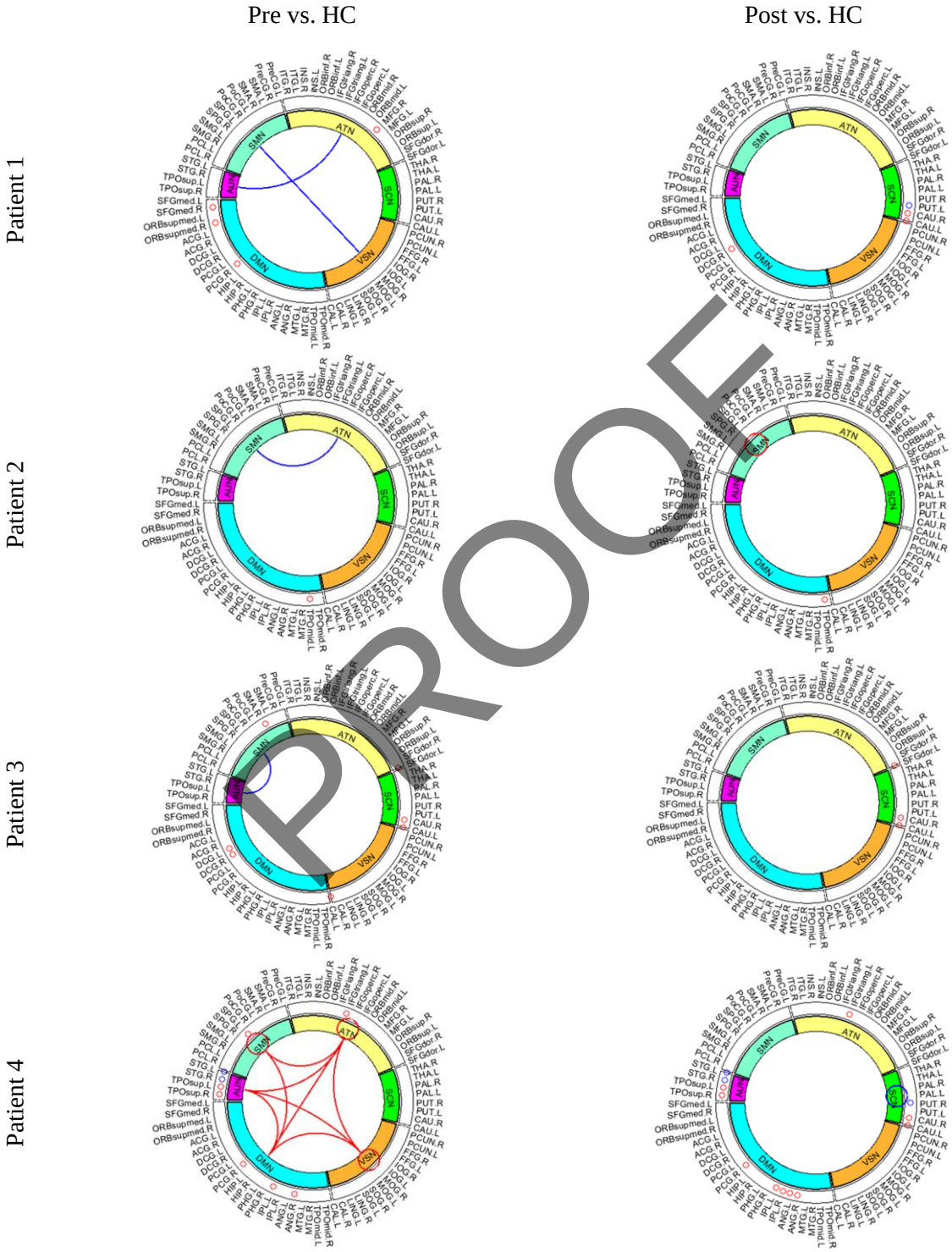


Figure 4. displays the differences in functional connectivity (FC) before and after neurofeedback in focal epilepsy patients compared to HC, based on the AAL atlas. The large red and blue colors represent an increase and decrease in FC within each cognitive network, respectively. The small colored circles depict the increased (red) or decreased (blue) nodal degree for specific nodes within each cognitive network, including the default mode network (DMN), attention network (ATN), sensorimotor network (SMN), visual network (VSN), subcortical network (SCN), and auditory network (AUN)

improvement in the patient's attention and response control (Figure 7).

When compared to the HC group before the therapy, the second patient showed a 3.2% reduction in functional connectivity between SMN-ATN. However, after NF therapy, the internal functional connectivity of the SMN increased compared to the HC group (Figure 4).

In the second patient, there was an increase in FA values in the fiber trajectories between DMN-AUN, SCN-AUN, and within the SCN compared to pre-treatment (CI = 95%) (Figure 5).

Patient 3

For patient 3, the visual and auditory attention scores were 58 and 78, respectively, before NF. After NF therapy, these scores increased to 79 and 85, respectively, indicating an improvement in the patient's attention and response control (Figure 7).

Compared to the HC group before treatment, the third patient showed a 25.8% reduction in functional connectivity between SMN-AUN. However, after NF therapy, there was no significant difference in these connections compared to the HC group (Figure 4).

In the third patient, an increase in FA values was observed between SCN-AUN (CI = 95%) (Figure 5).

Patient 4

For patient 4, the visual and auditory attention scores were 27 and 24 before NF, respectively. After SMR training, the scores increased to 59 and 88, respectively, indicating an improvement in the patient's attention and response control (Figure 7).

Compared to the HC group before treatment, the fourth patient showed an increase in functional connectivity between SMN-ATN (37%), SMN-DMN (33.4%), DMN-ATN (30.5%), AUN-ATN (26.5%), AUN-DMN (21.7%), AUN-VSN (18.9%), VSN-ATN (18.5%), and VSN-DMN (9.7%). Additionally, functional connectivity within SMN, ATN, and VSN was higher than the HC group. After NF therapy, these connections did not differ from the HC group, and only a 5.2% reduction in functional connectivity within the SCN was observed (Figure 4).

In the fourth patient, FA values in the fiber trajectories between multiple networks, including DMN-AUN, VIN-SCN, VIN-AUN, SCN-AUN, ATN-AUN, and SMN-AUN, as well as within the SCN, showed an increase compared to pre-treatment (CI = 95%) (Figure 5).

4. Discussion

The study described enrolled patients with drug-resistant focal epilepsy who underwent SMR training NF, evaluation of seizure activities, and estimation of brain functional and structural connectivity. This study is considered a pioneer multi-modal study that examines the brain networks as an outcome of NF therapy using fMRI and DTI assessments. The results showed that SMR training had immediate effects that were preserved in the 3-month follow-up. Although there was a positive trend in reducing seizures (35-100%), the lack of statistical significance in seizure frequency may be due to the fact that the recruited patients experienced only a few seizures per week before the therapy sessions. To be more confident about the treatment outcome, there is a need to recruit more patients with more frequent seizure experience.

The study also observed changes in brain network functional connectivity pre- and post-treatment, as well as an increase in fractional anisotropy values within the brain structural networks. In general, functional connectivity was improved and became similar to the HC group in all four patients. These findings suggest that SMR training NF may have a positive effect on brain network connectivity and may be a promising treatment option for drug-resistant focal epilepsy.

The study used a network-based approach to examine various network pattern changes due to the compensatory mechanism of NF. Prior to NF treatment, a decrease in functional association between the SMN and other networks such as VSN and ATN was observed in the patient group. In this regard, our findings are consistent with the results of the study of Fu *et al.* (2021). They reported a decrease in the association of SMN with attention networks (VAN and DAN) in patients with partial epilepsy [23].

This finding is also consistent with the findings of Besseling *et al.* (2013). They reported a decrease in functional connectivity between SMN and the left lower frontal gyrus (ATN) in children with partial epilepsy [24]. After NF treatment, these decreases

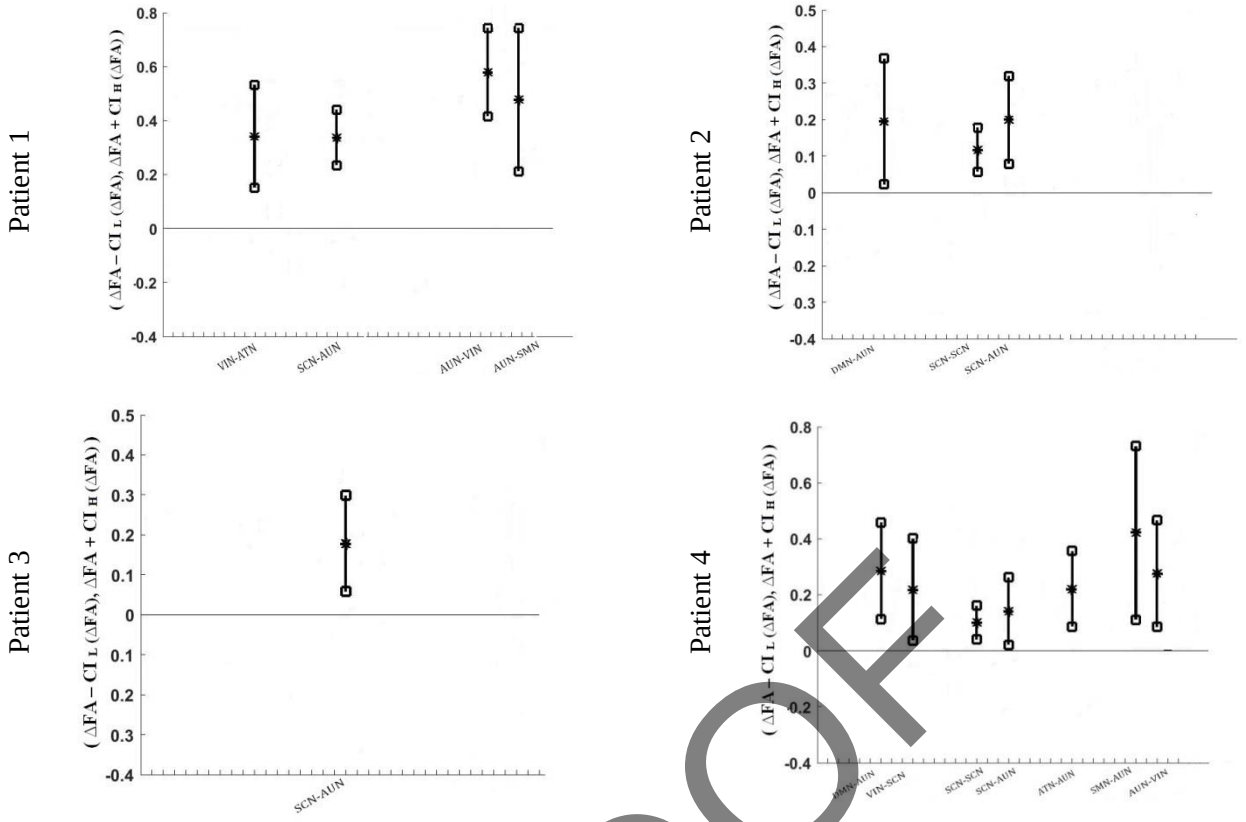


Figure 5. Illustrates the changes in mean fractional anisotropy (FA) in focal epilepsy patients' post- vs. pre-neurofeedback. The networks included in the analysis are the default mode network (DMN), attention network (ATN), sensorimotor network (SMN), visual network (VSN), subcortical network (SCN), and auditory network (AUN)

Table 3. presents the mean and standard deviation of IVA-2 scores at baseline and post-treatment for each participant

Patients	Mean ± SD (baseline)	Mean ± SD (post-treatment)	Cohen's d	MPI
1	90±26.4	101.5±17.13	0.52	11.33%
2	96±12.56	106.5±5.06	1.19	9.85%
3	78.25±19.7	89.25±15.37	0.62	12.32%
4	67.5±48.65	94±26.54	0.7	28.19%

in associations were eliminated, and there no longer was a significant difference observed between the two groups, which can be considered evidence of the effectiveness of SMR training NF on functional connectivity of SMN.

Another interesting finding was the decreased connectivity between AUN-ATN and AUN-SMN in two patients with temporal lobe epilepsy participating in the study. After NF, since this decrease was eliminated, there no longer was any difference seen between patients and the HC group.

The study also observed an increase in FA value between AUN and SCN in all patients after NF therapy, which was accompanied by an improvement in auditory attention after the intervention. Additionally, an increasing trend of FA value between SMN and other networks was observed, enhancing the sensorimotor rhythm of the brain, which increased structural fiber integrity between AUN and other networks in patients with temporal epilepsy and was associated with decreasing seizure frequency and severity in these patients.

The study evaluated the effects of NF on the patient's cognitive control and QEEG. The results showed an enhancement in sensorimotor rhythm after treatment, along with an increase in cognitive test scores. The IVA-2 test showed an elevation in all patients' scores, confirming the efficacy of the NF process, which was preserved throughout the follow-up period. Overall, the results of the study showed an improvement in integrated visual and auditory function after the therapy.

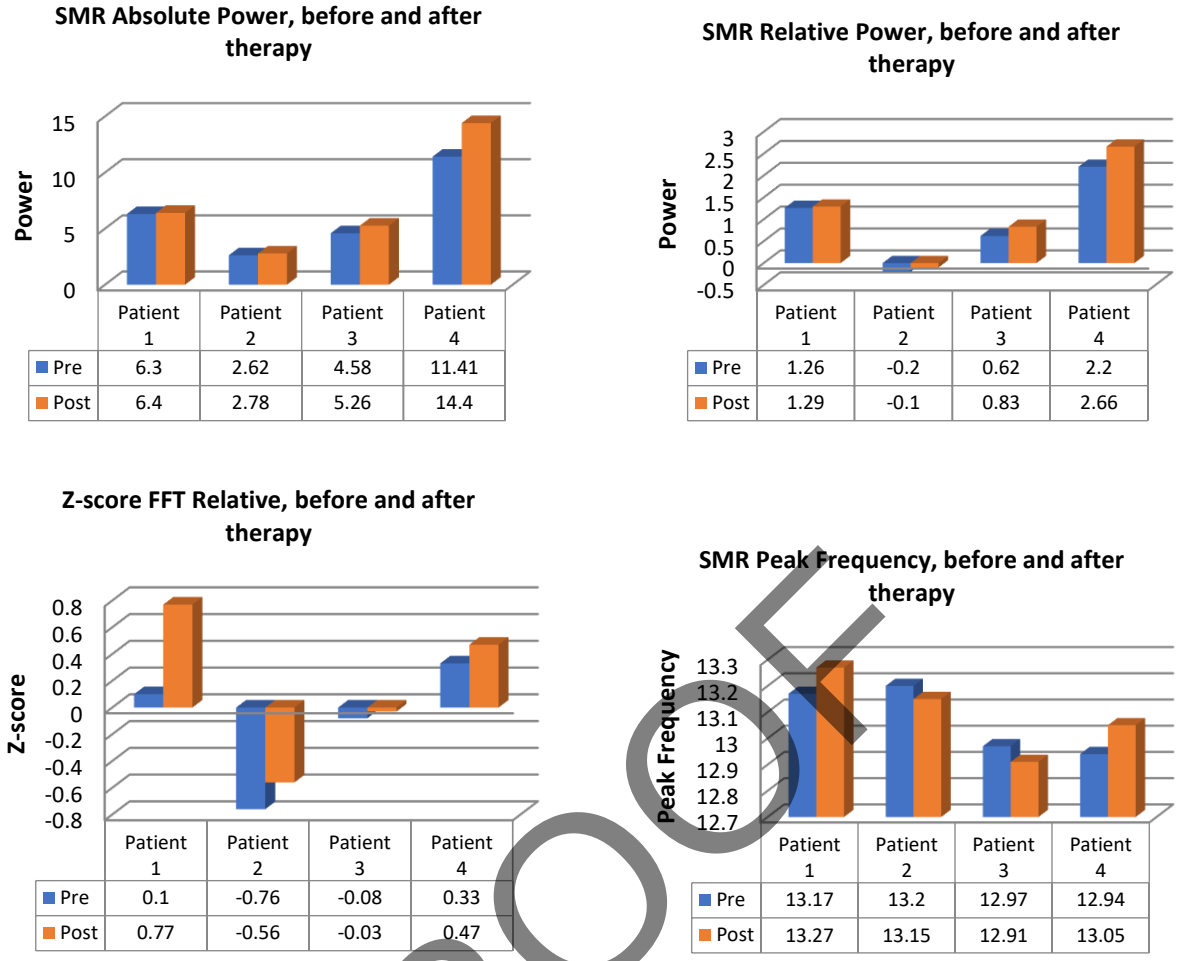


Figure 6. displays the absolute power (a), relative power (b) and its Z-score (c), and the peak frequency (d) of the sensorimotor rhythm (SMR) at the midline central zone (CZ) before and after the neurofeedback therapy

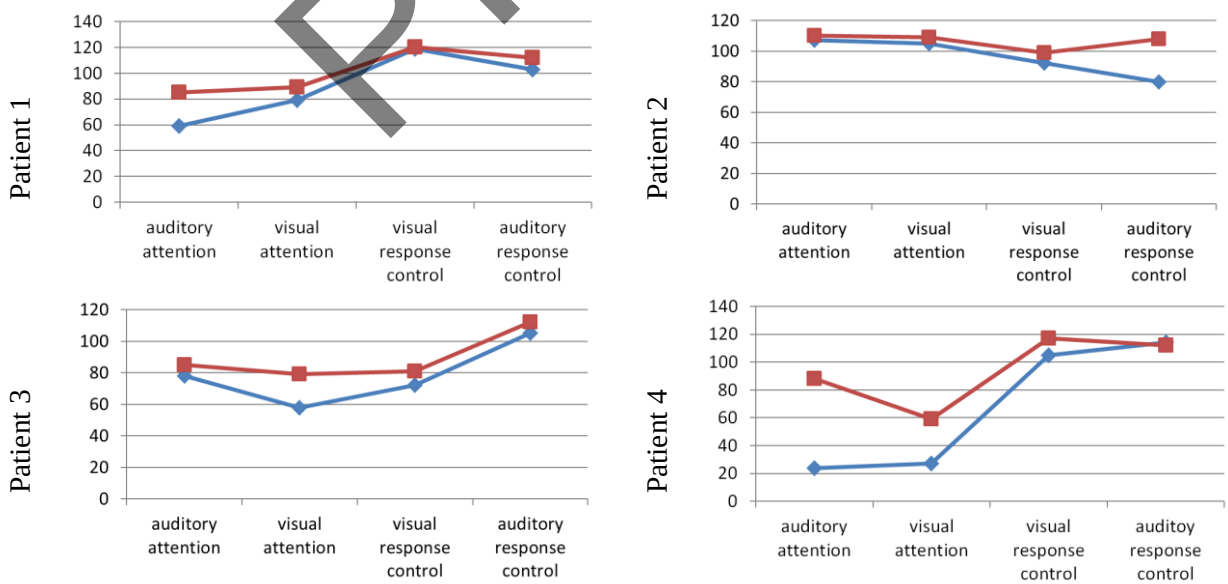
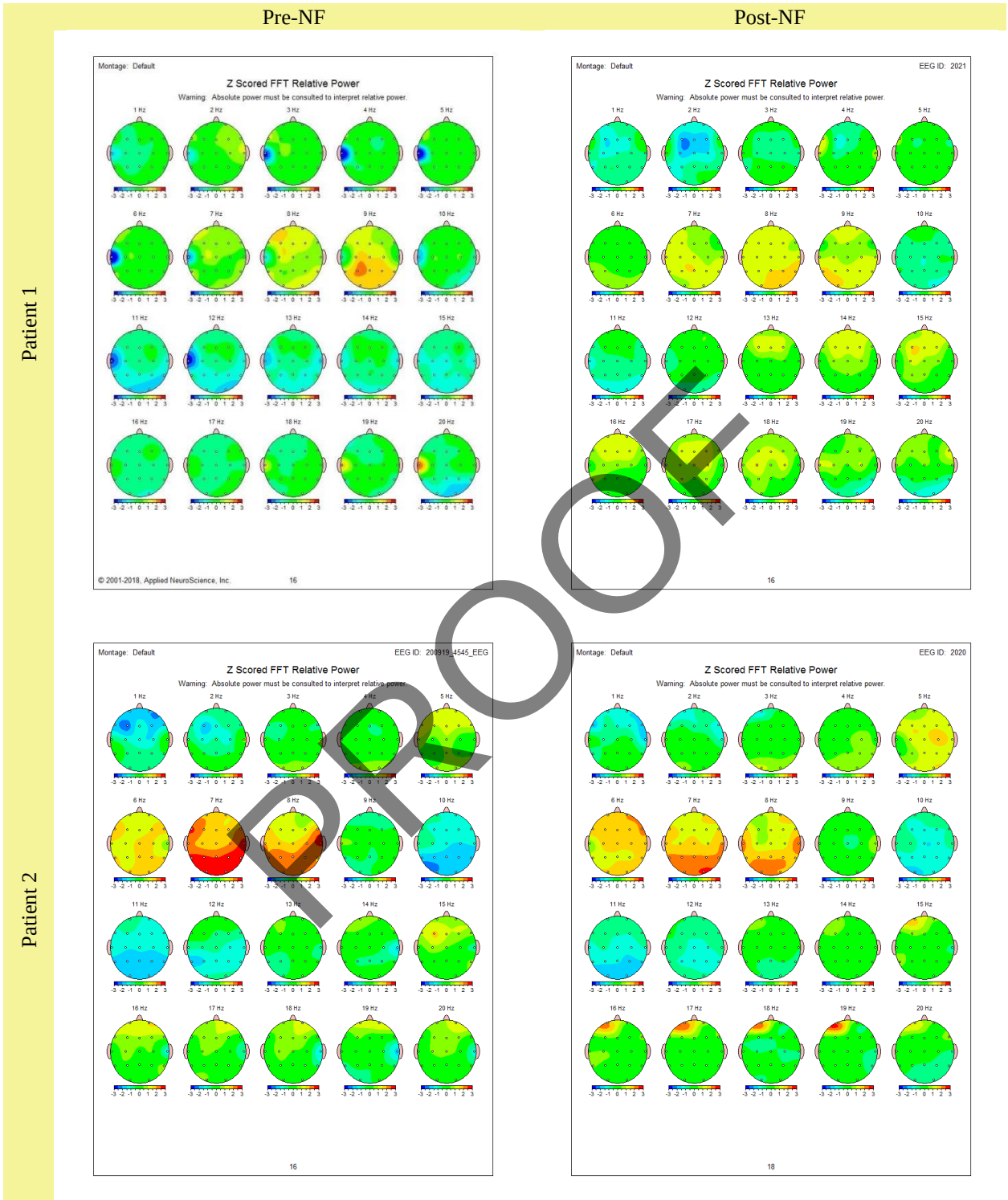


Figure 7. shows the changes in IVA-2 scores in focal epilepsy patients post- vs. pre-neurofeedback. The red and blue colors represent the post- and pre-neurofeedback scores, respectively

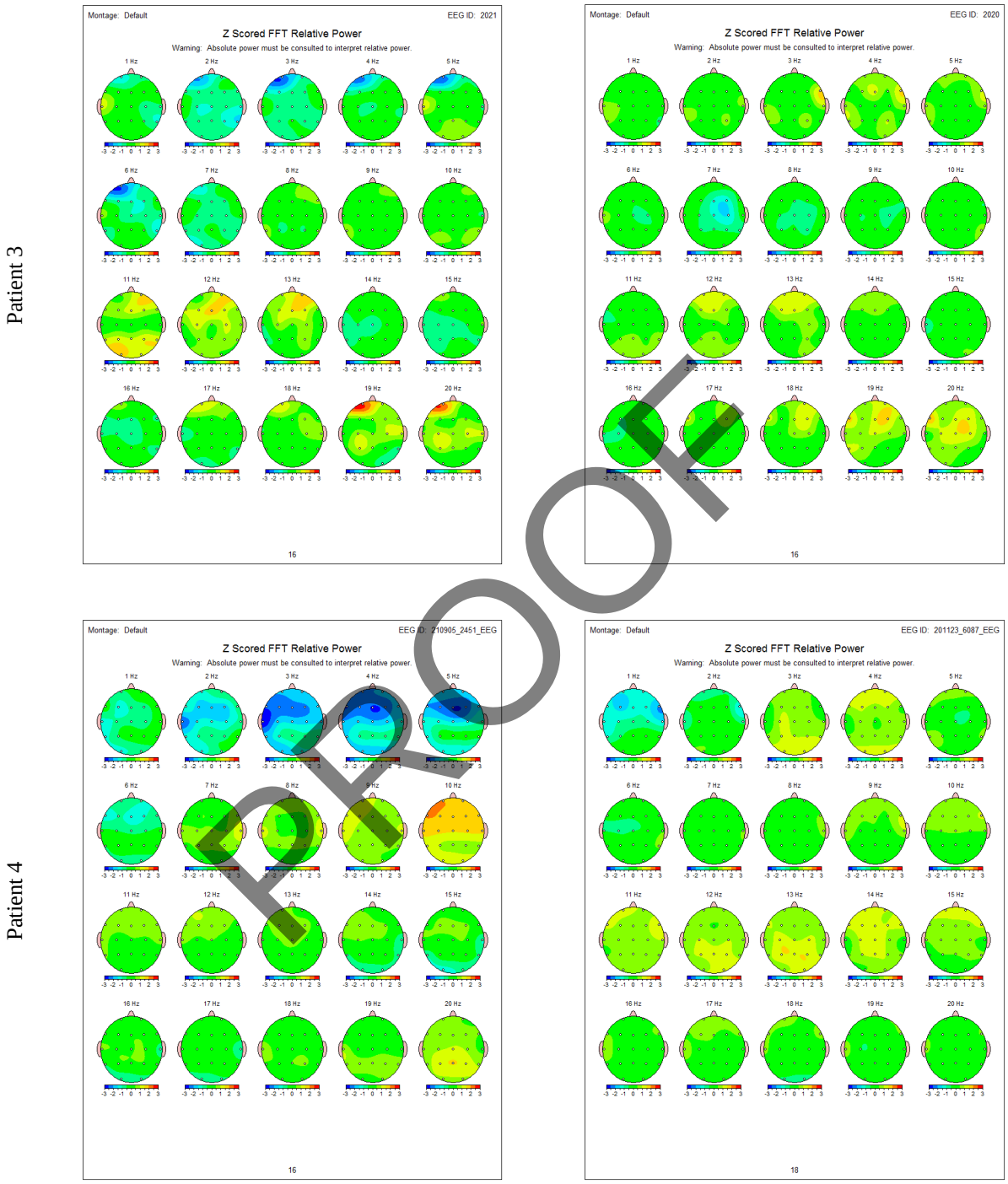
Figure 8. displays the QEEG (quantitative electroencephalography) of focal epilepsy patients before and after NF (neurofeedback) therapy. The hot colors (red, orange, and yellow) indicate higher than average values, while the cold colors (dark and light blue) indicate lower than average values



4.1. Limitations

The study has several limitations that need to be addressed. One significant challenge was the unpredictable nature of seizures in drug-resistant epilepsy patients, which caused some patients to drop out of the study. Additionally, some patients had cognitive impairment and difficulty with attention and concentration, leading to head movement and coil intolerance issues during imaging. The use of seizure diaries to track seizures may also be unreliable to some extent. The small sample size also limits the

Continuation of the Figure 8. Displays the QEEG (quantitative electroencephalography) of focal epilepsy patients before and after NF (neurofeedback) therapy. The hot colors (*red, orange, and yellow*) indicate higher than average values, while the cold colors (*dark and light blue*) indicate lower than average values



generalizability of the results, and a larger and adequately powered study is needed to confirm the findings. The study also suffered from a gender imbalance due to the withdrawal of two male participants. Future studies may focus on elucidating the exact cause of changes in global brain network organization following NF treatment, identifying relevant effect moderators, and characterizing functional and structural differences in focal subgroups. Personalized NF protocols, such as LORETA, may also be explored to improve treatment efficacy. Longitudinal studies and the systematic evaluation of data at more than two time points could also provide a better understanding of the relationship between epileptic seizures and changes in brain networks as a result of NF training. Finally, the study

was not designed to draw reliable conclusions regarding the long-term efficacy of NF in producing desirable and durable neurological effects.

5. Conclusion

The study has demonstrated that multi-modal imaging and clinical tests can be jointly used to evaluate the brain changes after a non-invasive intervention like NF. The dMRI and fMRI can be helpful in studying the neural bases of the NF mechanism and understanding its effectiveness in the brain of patients with focal epilepsy, and it is not sufficient to rely solely on the patient's EEG and self-declaration questionnaires. The pilot study's results provide evidence supporting further efforts to assess the efficacy of SMR training NF for focal epilepsy, and a larger patient cohort and a more comprehensive range of connectivity parameters will be required to gain meaningful insight into the use of NF for the treatment of focal epilepsy. In conclusion, the study has shown that a network-based approach using fMRI and DTI assessments can be used to evaluate brain network changes due to NF therapy, and further research is needed to explore the potential of NF as a treatment option for drug-resistant focal epilepsy.

Acknowledgments

The study was approved by the Institutional Review Board of Tehran University of Medical Sciences (Ethics code: IR.TUMS.MEDICINE.REC.1398.822), which ensured that the study was conducted in accordance with ethical principles and standards. All participants provided written informed consent, indicating that they fully understood the nature of the study, its potential risks and benefits, and their right to withdraw from the study at any time.

This work was funded and supported by the Medical School of the Tehran University of Medical Sciences [Grant Number: 98-3-101-45630] and the Cognitive Sciences & Technologies Council [Grant Number: 9228] between 2019 and 2022. The funding and support provided by these organizations were essential in enabling the study to be conducted, including the recruitment of participants, data collection, analysis, and interpretation, as well as the dissemination of the study's findings.

The authors have acknowledged the contribution of the Atieh Clinic Neuroscience Center in Tehran and their staff in clinical, neuropsychological, and neurophysiological data acquisition and neurofeedback therapy sessions. The Atieh Clinic Neuroscience Center's contribution was essential in enabling the study to be conducted and providing the necessary infrastructure and expertise to carry out the neurofeedback therapy sessions and data collection. The authors have also acknowledged the contribution of the National Brain Mapping Lab and their staff in MRI data acquisition. The acknowledgment of these contributions is important in recognizing the efforts and contributions of all individuals and institutions involved in the study's execution and ensuring that they are appropriately recognized and credited for their contributions.

References

1- Keun Tae Kim *et al.*, "Refining General Principles of Antiepileptic Drug Treatments for Epilepsy." (in eng), *Journal of clinical neurology (Seoul, Korea)*, Vol. 16 (No. 3), pp. 383-89, (2020).

2- Zhibin Chen, Martin J. Brodie, Danny Liew, and Patrick Kwan, "Treatment Outcomes in Patients With Newly Diagnosed Epilepsy Treated With Established and New Antiepileptic Drugs: A 30-Year Longitudinal Cohort Study." (in eng), *JAMA neurology*, Vol. 75 (No. 3), pp. 279-86, (2018).

3- R. Joshi, M. Tripathi, P. Gupta ,S. Gulati, and Y. K. Gupta, "Adverse effects & drug load of antiepileptic drugs in patients with epilepsy: Monotherapy versus polytherapy." (in eng), *Indian J Med Res*, Vol. 145 (No. 3), pp. 317-26, (2017).

4- Y. Nagai *et al.*, "Epileptic seizures are reduced by autonomic biofeedback therapy through enhancement of fronto-limbic connectivity: A controlled trial and neuroimaging study." (in eng), *EBioMedicine*, Vol. 27pp. 112-22, (2018).

5- R. Michaelis *et al.*, "Psychological treatments for people with epilepsy." (in eng), *Cochrane Database Syst Rev*, Vol. 10 (No. 10), p. Cd012081, (2017).

6- S. Åkerlund, E. Varkey, J. Klecki, J. Zelano, and E. Ben-Menachem, "Randomized controlled trial of moderate cardiovascular exercise for patients with drug-resistant epilepsy." (in eng), *Epilepsy Behav*, Vol. 124p. 108335, (2021).

7- J. B. Allendorfer and R. M. Arida, "Role of Physical Activity and Exercise in Alleviating Cognitive Impairment in People With Epilepsy." (in eng), *Clin Ther*, Vol. 40 (No .1), pp. 26-34, (2018).

8- N. Feter, R. Alt, C. A. Häfele, M. C. da Silva, and A. J. Rombaldi, "Effect of combined physical training on cognitive function in people with epilepsy: Results from a randomized controlled trial." (in eng), *Epilepsia,* Vol. 6 ١ (No. 8), pp. 1649-58, (2020).

9- G. Tan *et al.*, "Meta-analysis of EEG biofeedback in treating epilepsy." (in eng), *Clin EEG Neurosci,* Vol. 40 (No. 3), pp. 173-9, (2009).

10- JL Koberda and LC Frey, "Application of Z-score LORET A Neurofeedback in therapy of epilepsy." *Journal of Neurology and Neurobiology,* Vol. 1 (No. 1) , (2015).

11- M. B. Sterman and T. Egner, "Foundation and practice of neurofeedback for the treatment of epilepsy." (in eng), *Appl Psychophysiol Biofeedback,* Vol. 31 (No. 1), pp .21-35, (2006).

12- T. Egner and M. B. Sterman, "Neurofeedback treatment of epilepsy: from basic rationale to practical application." (in eng), *Expert Rev Neurother,* Vol. 6 (No. 2), pp. 247-57, (2006).

13- Gary H. Glover, "Overview of functional magnetic resonance imaging." (in eng), *Neurosurgery clinics of North America,* Vol. 22 (No. 2), pp. 133-vii, (2011).

14- Medana Luca, Christian Beckmann, Nicola De Stefano, Paul Matthews, and S. M. Smith, "FMRI resting state networks define distinct modes of long-distance interactions in the human brain." *Neuroimage,* Vol. 29pp. 1359-67, (2006).

15- Saba Amiri *et al.*, "Graph theory application with functional connectivity to distinguish left from right temporal lobe epilepsy." *Epilepsy Res,* Vol. 167, (2020).

16- Maryam Afzali *et al.*, "The sensitivity of diffusion MRI to microstructural properties and experimental factors." (in eng), *Journal of neuroscience methods,* Vol. 347pp. 108951-51, (2021).

17- E. Silvagni, A. Bortoluzzi, M. Borrelli, A. Bianchi, E. Fainardi, and M. Govoni, "Cerebral Microstructure Analysis by Diffusion-Based MRI in Systemic Lupus Erythematosus: Lessons Learned and Research Directions." (in eng), *Brain Sci,* Vol. 12 (No. 1), (2021).

18- Neil G. Simon and Michel Kliot, "Diffusion weighted MRI and tractography for evaluating peripheral nerve degeneration and regeneration." (in eng), *Neural regeneration research,* Vol. 9 (No. 24), pp. 2122-24 , (2014).

19- Yang Jiang, Reza Abiri, and Xiaopeng Zhao, "Tuning Up the Old Brain with New Tricks: Attention Training via Neurofeedback." (in English), *Frontiers in Aging Neuroscience,* Mini Review Vol. 9, (2017).

20- T. Egner and J. H. Gruzelier, "Learned self-regulation of EEG frequency components affects attention and event-related brain potentials in humans." (in eng), *Neuroreport,* Vol. 12 (No. 18), pp. 4155-9, (2001).

21- T. Egner and J. H. Gruzelier, "EEG biofeedback of low beta band components: frequency-specific effects on variables of attention and event-related brain potentials." (in eng), *Clin Neurophysiol,* Vol. 115 (No. 1), pp. 131-9, (2004).

22- Timothy P. Tinius, "The Integrated Visual and Auditory Continuous Performance Test as a neuropsychological measure." *Archives of Clinical Neuropsychology,* Vol. 18 (No. 5), pp. 439-54, (2003).

23- Cong Fu, Aikedan Aisikaer, Zhijuan Chen, Qing Yu, Jianzhong Yin, and Weidong Yang, "Different Functional Network Connectivity Patterns in Epilepsy: A Rest-State fMRI Study on Mesial Temporal Lobe Epilepsy and Benign Epilepsy With Centrotemporal Spike." (in English), *Frontiers in Neurology,* Original Research Vol. 122021-May-28, (2021).

24- René M. H. Besseling *et al.*, "Reduced functional integration of the sensorimotor and language network in rolandic epilepsy." *NeuroImage: Clinical,* Vol. 2pp. 239-46, (2013).

25- Christopher J. Ferguson, "An effect size primer: A guide for clinicians and researchers." 40 doi:10.1037/a0015808, *American Psychological Association,* US, (2009).

26- Gonzalo Rojas, Carolina Alvarez, Carlos Montoya Moya, Maria de la Iglesia Vaya, Jaime Cisternas, and Marcelo Gálvez, "Study of Resting-State Functional Connectivity Networks Using EEG Electrodes Position As Seed." *Frontiers in Neuroscience,* Vol. 1203/26 (2018).

27- M Barry Sterman, "Basic concepts and clinical findings in the treatment of seizure disorders with EEG operant conditioning." *Clinical electroencephalography,* Vol. 31 (No. 1), pp. 45-55, (2000).

28- Hengameh Marzbani, Hamid Reza Marateb, and Marjan Mansourian, "Neurofeedback: a comprehensive review on system design, methodology and clinical applications." *Basic and clinical neuroscience,* Vol. 7 (No. 2), p. 143, (2016).

29- Chao-Gan Yan, Xin-Di Wang, Xi-Nian Zuo, and Yu-Feng Zang, "DPABI: Data Processing & Analysis for (Resting-State) Brain Imaging." *Neuroinformatics,* Vol. 14 (No. 3), pp. 339-51, (2016).

30- N. Tzourio-Mazoyer *et al.*, "Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain." (in eng), *Neuroimage,* Vol. 15 (No. 1), pp. 273-89, (2002).

31- AJBSJJDK Leemans, Ben Jeurissen, Jan Sijbers, and Derek K Jones, "ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data ".in *Proc Intl Soc Mag Reson Med*, Vol. 17 (No. 1), p. 3537, (2009).

32- Josien PW Pluim, JB Antoine Maintz, and Max A Viergever, "Mutual-information-based registration of

medical images: a survey." *IEEE transactions on medical imaging*, Vol. 22 (No. 8), pp. 986-1004, (2003).

33- Alexander Leemans and Derek K Jones, "The B-matrix must be rotated when correcting for subject motion in DTI data." *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* ,Vol. 61 (No. 6), pp. 1336-49 , (2009).

34- H. X. Barnhart and D. P. Barboriak, "Applications of the repeatability of quantitative imaging biomarkers: a review of statistical analysis of repeat data sets." (in eng), *Transl Oncol*, Vol. 2 (No. 4), pp. 231-5 , (2009).

35- An Effect Size Primer: A Guide for Clinicians and Researchers." *Professional Psychology: Research and Practice*, Vol. 40pp. 532-38, (2009).