FUNCTIONAL CONNECTIVITY WITHIN LANGUAGE REGIONS IN STROKE PATIENTS WITH EXPRESSIVE APHASIA DURING REAL-TIME FUNCTIONAL MAGNETIC RESONANCE-BASED NEUROFEEDBACK TRAINING

A Thesis

Submitted By

TAPASI BRAHMA

For the award of the degree of

MASTER OF TECHNOLOGY, CLINICAL ENGINEERING

Jointly offered by

Indian Institute of Technology, Madras

Christian Medical College, Vellore

Sree Chitra Tirunal Institute for Medical
Sciences and Technology, Trivandrum

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CERTIFICATE

This is to certify that the thesis titled '**Functional Connectivity within Language Regions in Stroke Patients with Expressive Aphasia during Real-Time Functional Magnetic Resonance-based Neurofeedback Training**' being submitted by Tapasi Brahma to SCTIMST Trivandrum, for the award of degree of **Master of Technology** in **Clinical Engineering** jointly offered by IIT Madras, CMC Vellore and SCTIMST Trivandrum, is a bonafide record of research work done by him under our supervision. The contents of this thesis in full or in parts have not been submitted to any other Institute or University for the award of any degree or diploma.

The research had been carried out at Sree Chitra Institute of Medical Sciences and Technology, Trivandrum.

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LIST OF ABBREVIATIONS

AAL	Automated Anatomical Atlas
BCI	Brain Computer Interface
BF	BOLD Feedback
BL	Baseline
BOLD	Blood oxygenation level dependent
CO	Central Opercular
dHb	Deoxygenated Haemoglobin
EEG	Electro-encephalography
EES	Epidural Electrical Stimulation
EPI	Echo-Planar Images
FC	Functional Connectivity
FL	Frontal Language
fMRI	Functional Magnetic resonance imaging
FP	Frontal Polar
GLM	General Linear Model
Hb	Haemoglobin
HG	Heschl’s Gyrus
ICA	Independent Component Analysis
IFG	Inferior Frontal Gyrus
LFP	Local Field Potentials
MEG	Magneto-Encephalography
MRI	Magnetic Resonance Imaging
MTG	Middle Temporal Gyrus
PET	Positron emission tomography
PP	Planum Polare
PT	Planum Temporale
ROI	Region-of-interest

rs-fMRI	Resting-State fMRI
RT-fMRI	Real-time Functional MRI
rTMS	Repetitive Trancranial Magnetic Stimulation
SLT	Speech And Language Therapy
SP	Supra-Parietal
SPM	Statistical Parametric Mapping
STG	Superior Temporal Gyrus
TBV	Turbo Brain Voyager
tDCS	Transcranial Direct Current Stimulation
TE	Time to Echo
TL	Temporal Language
TP	Temporal Polar
TR	Repetition Time
UR	Up-Regulation
WAB	Western Aphasia Battery

ABSTRACT

Stroke is known to disrupt connectivity in the brain in addition to forming scars. This study analysed the application of a real-time fMRI neurofeedback training system, used for the rehabilitation of post-stroke Broca's aphasic patients. The study hypothesizes that with repeated sessions of the training, a rise in functional connectivity within the language regions will be observed for the aphasic patients.

The experiment was conducted on three groups of subjects: test patients, control patients, and normal participants, of size four each. The test and the normal groups underwent the training, whereas the control patients did not. In the training, the subject exercised language activity covertly to upregulate the Broca's area and amplify a feedback signal when it is correlated with the Wernicke's area, which is visually presented to the subjects as a bar graph to motivate them to improve their performance and stimulate neuroplasticity. As a measure of functional connectivity, Pearson's correlation coefficient was used. CONN toolbox was used to perform analysis of functional connectivity on the pre-processed data that was obtained on SPM8.

From the study, it was observed that after the training, for all the groups, a rise in functional connectivity was noticed mostly among the left hemispheric Regions of Interest (ROIs). While comparing the normal group with the test group, ROIs in the frontal polar region were noticed to have better functional connectivity in the former. While comparing the test group with the control group, ROIs in the supra parietal, and the right central opercular regions were found to have better functional connectivity in the former.

Stroke is a leading cause of disabilities, like aphasia, in the adult population of both developing and developed countries. This study can assist in the identification of regions in the brain that show an increase in functional connectivity among themselves in the process of rehabilitation of stroke patients suffering from expressive aphasia, which can further contribute to the design of rehabilitative training systems that are tuned to activate such regions in the brain.

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1.FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI)

fMRI provides the blood oxygen level dependent (BOLD) signal from the brain by the virtue of the difference in magnetization of oxygen-rich and oxygen-poor blood in various parts of the brain. This detects those areas of the brain which get activated in a particular cognitive or motor task. The hemodynamic response, which the modality provides is treated as a measure of the underlying neural activity. Software such as Statistical Parametric Mapping (SPM) and CONN are used for image processing to generate 3D images of brain activity, and for the analysis of functional connectivity in different areas of the brain.

1.2.STROKE AND APHASIA

A stroke is the rupturing or bleeding blood vessel in the brain, or the presence of an obstruction in the blood supply to the brain, which, thus, blocks blood and oxygen from reaching the tissues in the brain. Ischemic stroke occurs when blood vessels in the brain get narrowed or blocked, causing a severe reduction in the blood flow to brain tissues, whereas, hemorrhagic stroke occurs when a blood vessel in the brain leaks or ruptures. Stroke lesions often affect the Broca's area, the Wernicke's area and the connecting white matter tracts, which are the language centres of the brain. This may lead to aphasia. Based on the portion of the brain which is affected, aphasia can be categorized as Broca's aphasia, Wernicke's aphasia, or conduction aphasia. In acute stroke therapy, blood flow to the brain is restored and neuronal function recovers in the penumbral region. Additional recovery is caused by neuroplasticity, in which either the ipsilateral areas surrounding the lesion or the contralateral homologous regions take over the function of the lesioned portion. This plasticity can bring about changes in functional connectivity in the brain, which is defined as the statistical dependence between two regions in the brain.

1.3. RT-fMRI BASED NEUROFEEDBACK SYSTEM FOR NEUROREHABILITATION

The real-time-fMRI-based neurofeedback system, used in this study, produced feedback as per the BOLD activity from particular regions of the brain. MR images were acquired in quick succession, after which, the acquired images were pre-processed for the correction of artifacts. Regions-of-interest (ROIs) were identified based on anatomical landmarks and BOLD activity patterns, and the feedback was presented to the participants using software such as Presentation. A neurofeedback loop was used to conducted this operant training of subjects, which is the presentation of rewards or punishments upon any particular behaviour of the subjects, to induce voluntary control of language activities.

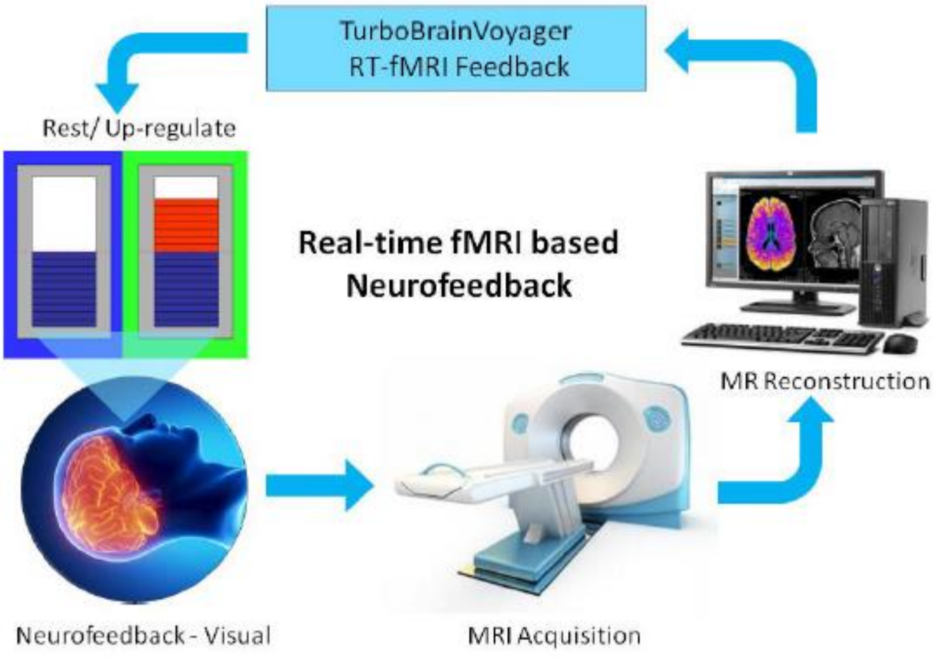


Fig 1: Architecture of the Real-time fMRI-based Neurofeedback System

(Source: Sreedharan et al., 2019)

1.4.LANGUAGE REGIONS IN THE BRAIN

The Broca’s and the Wernicke’s areas are structurally linked via the arcuate fasciculus. Recent research has proven that the two areas are connected through an indirect pathway, through the inferior parietal cortex. Additionally, ventral pathways connect the anterior portion of the inferior frontal gyrus to the temporal cortex via the extreme fibre capsule system and the frontal operculum to the anterior temporal cortex via the uncinate fasciculus.

Along with affecting the functioning of the lesioned brain region, stroke lesions also obstruct the activities in those regions which are dependent on the excitatory inputs from the lesioned region. It has been hypothesized that recovery of language in stroke survivors is possible by the reactivation of the deafferented brain regions with alterations in pathways. According to another hypothesis, undamaged regions may acquire the functions of the affected regions. Such recovery modes can result in new pathways and be reflected as new and stronger connections in the functional connectivity network.

1.5.CONN: FUNCTIONAL CONNECTIVITY TOOLBOX

CONN is a MATLAB/SPM-based cross-platform software used for the computation, analysis, and display of functional connectivity in fMRI (fcMRI). CONN has applications for both resting-state data (rsfMRI), and task-related designs. It covers the entire pipeline from raw fMRI data to hypothesis testing. The processing and analysis steps in CONN are as follows:

1. Importing DICOM, ANALYZE, and NIfTI functional and anatomical files, either raw or partially/fully preprocessed
2. Preprocessing pipelines of the functional and anatomical volumes, which includes susceptibility distortion correction, motion correction/realignment, slice-timing correction, outlier identification, coregistration, tissue-class segmentation, MNI-normalization, and smoothing
3. Control of residual physiological and motion artifacts, which includes scrubbing, aCompCor, ICA-based denoising, Global Regression, band-pass filtering
4. Quality control procedures, like FC histogram plots, BOLD signal carpetplots
5. Connectivity analyses, like Seed-Based Correlations, ROI-to-ROI analyses, complex-network analyses, generalized Psycho-Physiological Interaction model, Independent Component Analyses (ICA)
6. Group- and population-level inferences and models, including ANOVA, regression, univariate and multivariate statistics

1.6.HYPOTHESIS AND OBJECTIVES OF THE STUDY

The hypothesis of the study is that with neurofeedback of the BOLD signals from specific language regions in the brain identified with the aid of functional localizer experiments, one in and around the Broca's area and another in and around the Wernicke's area, the post-stroke patients with aphasia will be capable of increasing the functional connectivity in these regions with repeated exercise. This neurofeedback training and the accompanying up-regulation in the language regions are believed to assist the recovery of language through learning and neuroplasticity with better functional connectivity among these language regions.

The objectives of the study are:

1. to study intramodular ROI to ROI functional connectivity in test, normal, and control groups of subjects, under various conditions, measured by Pearson's correlation coefficient and presented in the form of connectivity matrices
2. to perform one-sample and two-sample t-tests on the values obtained in the above step, for intra-group, and inter-group comparisons of subjects, respectively, in order to establish the statistical significance of the obtained results
3. to study intramodular ROI to ROI functional connectivity in the four test patients, individually, measured by Pearson's correlation coefficient and presented in the form of connectivity matrices, and compare the changes in functional connectivity at the final session of training over the first session

CHAPTER 2

LITERATURE REVIEW

2.1. STROKE AND APHASIA

Stroke causes focal or global disruption in cerebral function. It is one of the most common causes of disabilities, like aphasia in the adult population worldwide.

Aphasia is an acquired communication disorder that damages a subject’s capacity to understand, produce and use language. It is the commonest disability in stroke survivors (Plowman et al., 2012).

Aphasia can be classified based on it’s symptoms. In Broca’s aphasia, also called expressive aphasia the ability to express speech is hampered. There is a limitation on vocabulary and the generation of speech gets arduous and non-fluent. Lesions in the case of Broca’s area are present in the inferior frontal gyrus, the lower portion of the precentral gyrus, and the insular and the opercular regions (Hojo et al., 1985; Plowman et al., 2012).

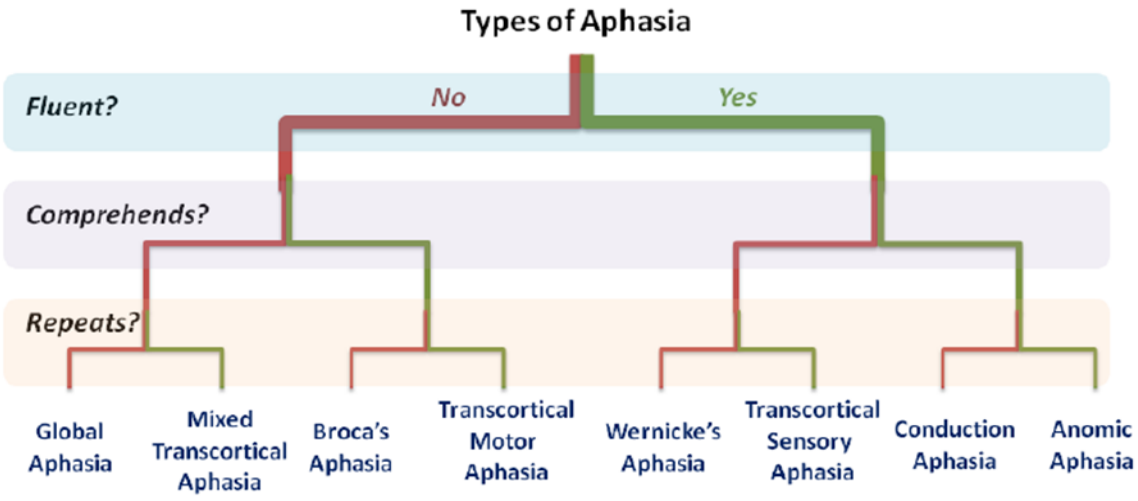


Fig 2: Types of Aphasia and their classification

(Source: www.aphasia.org)

In Wernicke’s aphasia, the comprehension of speech is compromised. Since speech may be fluently generated in the case of Wernicke's aphasia, it is also called 'fluent aphasia’. Reading and writing skills are severely disturbed. Lesions in this form of aphasia are present in the posterior part of the superior temporal gyrus, and the inferior parietal regions comprising of the angular gyrus and supramarginal gyrus (Hart and Gordon, 1990; Kertesz et al., 1993).

2.2. LANGUAGE AREAS OF THE BRAIN

According to the classical model of language organization, language function is localized in the left frontal and temporal lobes of the brain. The Broca’s area in the left frontal lobe is responsible for the expression of language and the Wernicke’s area in the left temporal lobe, for it’s comprehension. However recent research has presented results that do not align with this classical model. The existence of left hemispheric temporoparietal language areas exterior to the conventional Wernicke area, namely, in the middle temporal, the fusiform, the inferior temporal, and the angular gyri, and the existence of left prefrontal language areas exterior to the conventional Broca area have been discovered (Binder et al., 1997).

A study using Positron Emission Tomography showed that auditory word forms are processed in the left temporoparietal cortex, visual words are formed in the left extrastriate cortex, semantic associations encompass the left ventral prefrontal cortex, and word generation involves the dorsolateral prefrontal cortex. In the study, it was also found that complex language functions are not confined to specific brain regions but are spread across networks of regions with each area making a particular contribution to the task which depends on its connections to other areas (Petersen et al., 1988; Price, 2012).

Recently, research has revealed the participation of the right hemisphere in prosody. For example, the right superior temporal region gets stimulated during the prosodic processing of speech, that is, the detection of variations in the frequency, rhythm, and intonation in speech (Bookheimer, 2002). Further, during the processing of metaphors when the semantic content of sentences is challenging to follow, activation in the left and right pars opercularis and orbitalis increases (Price, 2010).

Earlier, the study of brain connectivity was dependent upon the dissection of the cadaver brain. However, currently, structural imaging techniques such as Computed Tomography and

Magnetic Resonance Imaging make it possible to study brain anatomy without involving post-mortem dissection. The language-relevant cortex consists of Broca’s area that is in the inferior frontal gyrus (IFG), Wernicke’s area that is in the superior temporal gyrus (STG), with parts of the middle temporal gyrus (MTG) and the inferior parietal and angular gyrus in the parietal lobe. As per the cytoarchitectonic classification of Brodmann Broca’s area, consists of Brodmann Area 44 (BA44), which is the pars opercularis, and the BA45, which is the pars triangularis of the IFG. BA44 connects to the temporal cortex via a dorsal pathway consisting of the arcuate fasciculus and the superior longitudinal fasciculus.

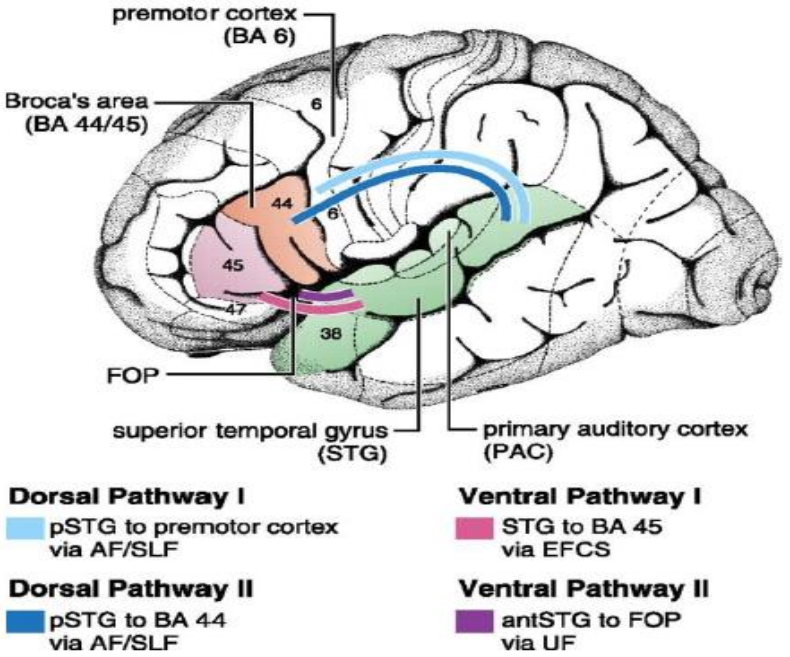


Fig 3: Structural connectivity among language cortices

(Source: Friederici, 2011)

The BA45 connects to the temporal cortex through the extreme fiber capsule system. It has been found that the frontal operculum connects to the anterior temporal cortex via the uncinate fasciculus. Additional to these long-range connections, two short-range pathways within the temporal cortex, one from the Heschl’s gyrus (HG) to the planum polare and anterior STG via a rostral fiber pathway, and the other from HG to the planum temporale and posterior STG via a caudal fiber pathway have been found (Friederici, 2011).

2.3. METHODS OF CONNECTIVITY ANALYSIS

By definition, functional connectivity (FC) is the statistical dependence between spatially remote neurophysiological events (Friston et al., 1993; Lee et al., 2003). As opposed to anatomical connectivity that defines the physical connections among regions in the brain, functional connectivity inspects interactions among regions in the brain at a macroscopic level, using modalities like electro-encephalography (EEG), magneto-encephalography (MEG), local field potentials (LFP), positron emission tomography (PET), functional MRI, etc (Boccaletti et al., 2006). In comparison with other imaging modalities, functional MRI is a non-invasive and yet in vivo depiction of brain state with good spatial resolution. Toolboxes are available for performing this analysis, such as CONN, and FSL (Kawabata Duncan et al., 2013; Pravata et al., 2011).

2.4. REHABILITATION OF PATIENTS WITH APHASIA

Recovery from stroke is a complicated process that commences with spontaneous recovery in the acute phase due to the reperfusion of hypoxic tissue in perilesional regions with a lessening of oedema, and is followed by a process that is dependent on learning that extends to the sub-acute and chronic phase (Langhorne et al., 2011). The recovery saturates in the chronic stage, which does not usually get ameliorated without intervention. The learning-dependent recovery can be attributed to the capacity of the human brain to learn and adapt which can be selectively stimulated by speech and language therapy (SLT) (Albert and Kesselring, 2011). The objective of clinical neurorehabilitation is to build a recurring learning condition and a motivating environment. Concentrated SLT for an average of about 8 hours per week and continued for 11 weeks has shown to produce substantial effects.

Pharmacological therapy with the aid of drugs is another domain of clinical application and research. Electromagnetic stimulation is also in use to selectively alter the excitability of brain regions to excite or inhibit certain regions to promote recovery. Transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) are believed to assuage the shortfall in the language in aphasic patients. Epidural electrical stimulation (EES) is also used to selectively excite brain tissue (Albert and Kesselring, 2011; Berthier and Pulvermüller, 2011).

Recovery in stroke can also be attributed to neuroplasticity (Berthier and Pulvermüller, 2011). Morphological changes such as synaptic plasticity and sprouting of axons and dendrites can augment neuroplasticity (Albert and Kesselring, 2011). Mechanisms of recovery supported by the left hemisphere have been observed to result in decent recovery for small lesions, whereas for a more extensive injury right hemisphere is involved (Crosson et al., 2007).

Many studies have shown that involvement of left hemisphere perilesional results in better recovery and that of right hemisphere leads to poor recovery of language. It is believed that the right hemispheric recruitment is not due to language recovery, rather it is due to the loss of inhibition by the left hemisphere (Crosson et al., 2007; Thompson and den Ouden, 2008). However, in healthy subjects, right-hemispheric involvement is known to be significant in language recovery. This hints towards the hypothesis that in the case of stroke in the left hemisphere, right-hemispheric regions may take over the language function. Hence the selection of the region to stimulate (or inhibit) is a subject of research and requires elucidation of the underlying neural recovery mechanisms and its correlation to lesion size, location, and extent (Crosson et al., 2007; Thompson and den Ouden, 2008).

In a study, it was found that during normal narrative speech comprehension, the left anterolateral superior temporal cortex exhibited functional connectivity with the left anterior basal temporal cortex, left inferior frontal gyrus, and homotopic cortex in the right anterolateral superior temporal cortex, in the positive direction. In aphasic patients, selective disruption of the standard functional connectivity between the left and the right anterolateral superior temporal cortices was seen (Warren et al., 2009). It was found that shortfall in auditory single word and sentence comprehension is due to interruption in left-right anterolateral superior temporal cortical connectivity, with local activation in the anterolateral superior temporal cortex. Another study shows that disturbance in inter-hemispheric connectivity in the somatomotor network and the dorsal attention network has a stronger relation to behavioral impairment in those domains than is intrahemispheric connectivity within either the lesioned or unaffected hemisphere (Carter et al., 2012). Another study showed that direct training effects resulted in increased functional connectivity for regions involved in abstract word processing (Sandberg et al., 2015).

2.5. REAL-TIME FUNCTIONAL MAGNETIC RESONANCE IMAGING

Functional magnetic resonance imaging (fMRI) has become a popular tool in neuroimaging since its advent in the early 1990s in both clinical and research domains. fMRI provides very good spatial resolution and relatively better temporal resolution when compared to other modalities. fMRI is a measure of neural activity and relies on the fact that when neurons in a particular region become active, blood flow towards that region escalates.

In 1890, two physiologists Roy and Sherrington showed that local neuronal activity is associated with regional changes in cerebral blood flow and metabolism (Roy and Sherrington, 1890). In 1936 magnetic properties of blood were discovered by Linus Pauling and Charles Coryell. They noticed that the magnetic properties of a blood cell (haemoglobin) are dependent on whether it has an oxygen molecule, since blood with oxygen has no magnetic moment, whereas, blood without oxygen has a significant magnetic moment (Pauling and Coryell, 1936). The magnetic susceptibility of dHb is about 20% more than that of Hb. This magnetic susceptibility modifies the rate of spin dephasing. In 1992 Kwong demonstrated the first BOLD-contrast fMRI in the human visual cortex. Kwong also described that variations in BOLD signal corresponded to changes in blood flow (Kwong et al., 1992).

There are two paradigms in experiments in fMRI: task-based fMRI, and resting-state fMRI. In task-based studies, subjects are required to perform a motor or cognitive task, while scanning is in progress. There are alternate blocks of task and rest in this paradigm. In resting-state fMRI, no task is carried out by subjects. Subjects rest inside the scanner, getting disengaged from any active task, while not falling asleep.

For real-time applications, operations such as comparison of active state and baseline state using differences in mean voxel activity, and correlation analysis can be performed (Sitaram et al., 2008). A high correlation coefficient suggests significant activation.

fMRI is being applied as a neurofeedback tool in the form of a real-time fMRI (RT-fMRI) based neuro-feedback system. Research has shown that a Brain-Computer Interface (BCI) can be designed using RT-fMRI (Yoo et al., 2004). A disadvantage of RT-fMRI-based BCI is that it is not a portable system and that MRI scanning is very expensive. In the neurofeedback loop, maps of neural activities can be presented to the subject being scanned (Caria et al., 2011).

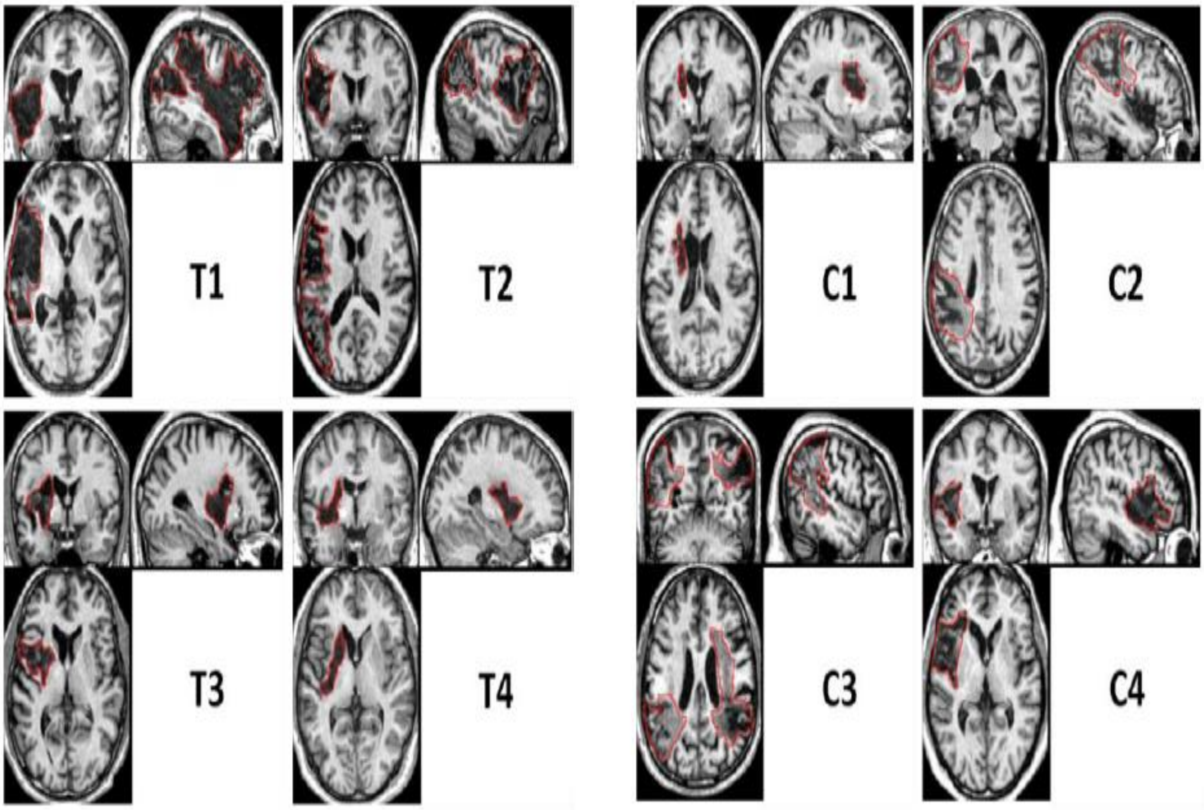
With the aid of RT-fMRI, self-regulation of a particular location in the cortical or subcortical areas of the brain is possible. Regulation of BOLD activity by neurofeedback may take place both in the positive (up-regulation) and negative directions (down-regulation). On studying the effects of self-regulation of the right IFG which is associated with prosody and language processing, it was found that self-regulation of the right BA45 can be learned upon several sessions of training. As a result of the same, a significant development in accuracy can be noticed in the identification of emotional prosodic intonations but not in syntactic processing (Rota et al., 2009). Some studies show that the ability to self-regulate BOLD activity without feedback can persist for a long duration such as more than six months after training (deCharms et al., 2004; Kotchoubey et al., 1997).

CHAPTER 3

METHODOLOGY

3.1. SELECTION OF SUBJECTS

This study has been performed on the fMRI data collected by the Department of Imaging Sciences and Interventional Radiology, SCTIMST, and the Department of Medical Device Engineering, Biomedical Technology Wing, SCTIMST. It is also inspired by the study previously conducted on the data from December 1, 2012, to May 16, 2015 (Sreedharan et al.). Recruitment of four test patients and four control patients was done during a period between six weeks to six months after the stroke. The subjects were diagnosed only with expressive aphasia and their comprehension was relatively unaffected so that they could perform in the study as per instructions. Additionally, four healthy volunteers were also recruited, who were referred to as the normal group. The study was approved by the ethics committee of the institute and was carried out after obtaining informed consents from the subjects.



(a) Test Group (b) Control Group

Fig.4: Size and location of stroke lesions in patients

(Source: Sreedharan et al., 2019)

The inclusion criteria were:

(i) patient with age greater than 18 years,
(ii) the primary language of communication is Malayalam,
(iii) the patient should be right-handed according to Edinburgh handedness inventory,
(iv) the patient should be willing to co-operate for the study,
(v) the patient should be motivated for speech therapy,
(vi) an interval of 6 weeks to 6 months post-stroke, and

(vii)the patient should be diagnosed with expressive aphasia

The exclusion criteria are:

(i) patients having receptive aphasia,
(ii) patients possessing pacemaker or other MRI-incompatible metallic implants
(iii) claustrophobic patients, and
(iv) patients using spectacles for myopia

An assessment of the subjects was first conducted using a Western Aphasia Battery (WAB) test which was carried out in Malayalam. The Western Aphasia Battery was intended to offer a means of evaluating the clinical properties of language function, like spontaneous speech, naming, repetition, reading, writing, apraxia.

After the recruitment of subjects, six RT-fMRI sessions were conducted for test patients and two session were conducted for the control patients. The participants were provided with instructions about the tasks to be performed which included distinguishing the rest block from the up-regulation block, and responding to a picture-naming activity by button pressing. The subjects were also asked to adopt an appropriate method for up-regulation of language to raise the activation levels being presented on a screen, like having a conversation, making a speech, reciting a poem, etc.

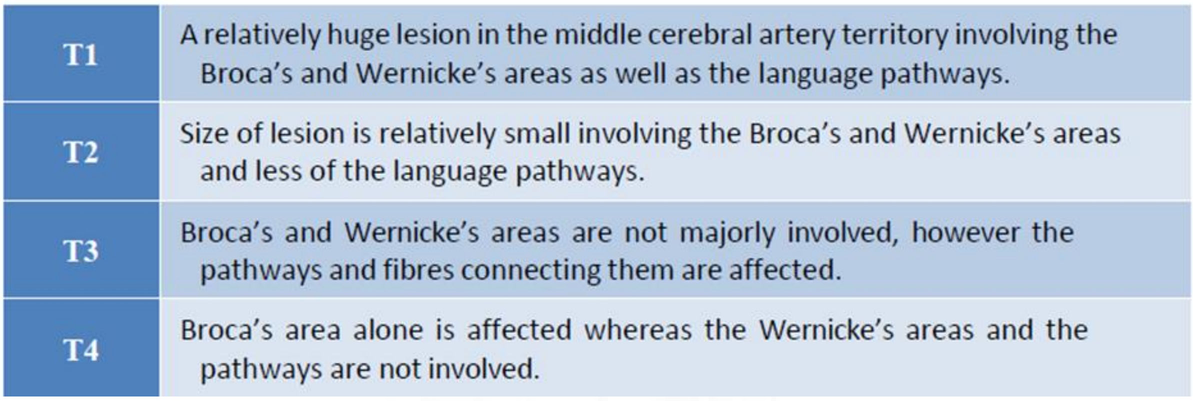
Test Patient	Age	Sex	Interval from ictus to session (weeks)						Neurological Deficits
			S1	S2	S3	S4	S5	S6	
T1	34	M	22	27	28	33	34	34	Right upper and lower limb weakness, slurring of speech
T2	35	M	8	9	10	11	12	13	Right upper and lower limb weakness, deviation in angle of mouth, inability to speak
T3	40	M	7	9	10	11	12	13	Right upper and lower limb weakness, inability to speak
T4	18	M	15	16	17	18	19	20	Right upper and lower limb weakness, inability to speak
Control Patient	**Age**	**Sex**	**Interval from ictus to session (weeks)**						**Neurological Deficits**
			S1	**-**	**-**	**-**	**-**	**S2**	
C1	40	M	15	-	-	-	-	19	Right upper and lower limb weakness, slurring of speech
C2	68	M	6	-	-	-	-	10	Right upper and lower limb weakness, inability to speak
C3	59	M	16	-	-	-	-	28	Right upper and lower limb weakness, slurring of speech
C4	57	F	12	-	-	-	-	14	Slurring of speech, no weakness

Table 1: The test and control groups

Majorly, the left hemisphere in the subjects was affected in stroke.

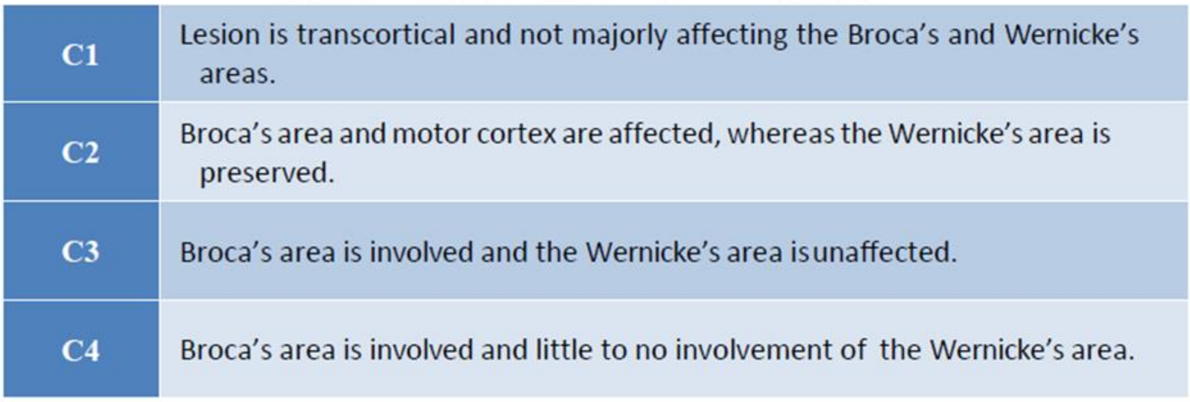
Normal Subject	Age	Sex	Interval between sessions (weeks after S1)					
			S1	S2	S3	S4	S5	S6
N1	27	M	0	4	5	8	9	12
N2	36	M	0	2	5	6	37	41
N3	28	M	0	4	5	6	37	43
N4	28	M	0	1	3	4	7	35

Table 2: The normal group



T1	A relatively huge lesion in the middle cerebral artery territory involving the Broca's and Wernicke's areas as well as the language pathways.
T2	Size of lesion is relatively small involving the Broca's and Wernicke's areas and less of the language pathways.
T3	Broca's and Wernicke's areas are not majorly involved, however the pathways and fibres connecting them are affected.
T4	Broca's area alone is affected whereas the Wernicke's areas and the pathways are not involved.

Table 3: Description of stroke lesions in test patients



C1	Lesion is transcortical and not majorly affecting the Broca's and Wernicke's areas.
C2	Broca's area and motor cortex are affected, whereas the Wernicke's area is preserved.
C3	Broca's area is involved and the Wernicke's area isunaffected.
C4	Broca's area is involved and little to no involvement of the Wernicke's area.

Table 4: Description of stroke lesions in control patients

3.2. ARCHITECTURE OF THE REAL-TIME fMRI NEUROFEEDBACK SYSTEM

A 1.5T MR scanner (Siemens Avanto) was used to scan echo-planar images (EPI) maintaining a repetition time (TR) of 1.5 seconds. The brain was imaged with 16 slices of 64 x 64 pixels acquired in a single TR. The voxel size was 3.28x3.28x6mm3 and structural images having a resolution of 1x1x1.1mm3 were obtained before the fMRI sessions to locate ROIs in the brain and to lay functional maps on the brain structure. The TR of functional EPIs was fixed at 1.5s and a time to echo (TE) of 45ms was chosen. Each scan was retrieved from the MR workstation after reconstruction and sent to the BCI computer. The real-time transfer was configured with the aid of an interface called the Ideacmdtool.

Turbo Brain Voyager (TBV) functioned as the core of the RT fMRI-based neurofeedback loop. The MR images were processed, and their statistical analysis was carried out using a general linear model (GLM) (Friston et al., 1994). The BCI makes use of two ROIs which were delineated with the help of a functional localizer task. The functional localizer task was conducted in each session to locate the target ROIs to be used for neurofeedback training. It consisted of a word generation task in which the participants were shown a letter in Malayalam and they had to continuously generate words starting with that letter for 15 seconds (10 scans). This is iterated five times with intermittent rest blocks of equal duration. The functional localizer was processed and the significantly activated clusters were generated instantly after the functional localizer run. The cluster in and around the Broca's area (IFGtri, IFGoper) is selected as ROI1 and the cluster in and around the Wernicke's area (pSTG) is selected as ROI2.

The RT-fMRI Neurofeedback training system had the following components:

1) MR scanner

2) LAN connections between MRI workstation, TBV computer, and presentation computer

3) TBV computer running the RT-fMRI software and scripts generating feedback values

4) Presentation computer, generating visuals of feedback response and recording patient response during the picture naming tasks

5) Overhead projector projecting the visual feedback from either the presentation computer or TBV computer to the screen within the MRI room

6) Avotec based fMRI system with MR compatible response boxes, for interfacing the fMRI trigger pulses generated before each scan, and the button responses as serial inputs to the presentation computer

The first ROI is chosen in the activated region in the IFG, the second in the STG, both in the left hemisphere. For one test patient (T1) the regions were chosen in the right hemisphere as there was no activation in the left hemisphere. A MATLAB script was run which used the mean of the latest three BOLD activity levels of the first ROI, and the latest ten values of both the first and the second ROIs' time series to process the correlation to get the BOLD feedback (BF) as follows (Equation 1):

$$BF = (\overline{RoI_1}) * \{1 + corr(RoI_1, RoI_2)\} \quad - \quad (1)$$

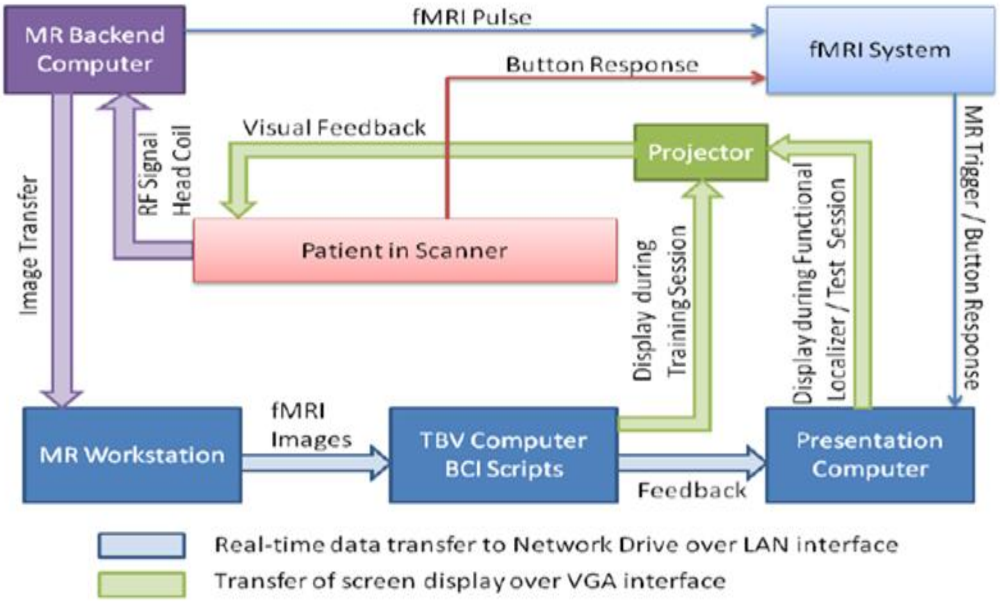


Fig.5: Block Diagram of RT-fMRI Neurofeedback Training System

(Source: Sreedharan et al., 2019)

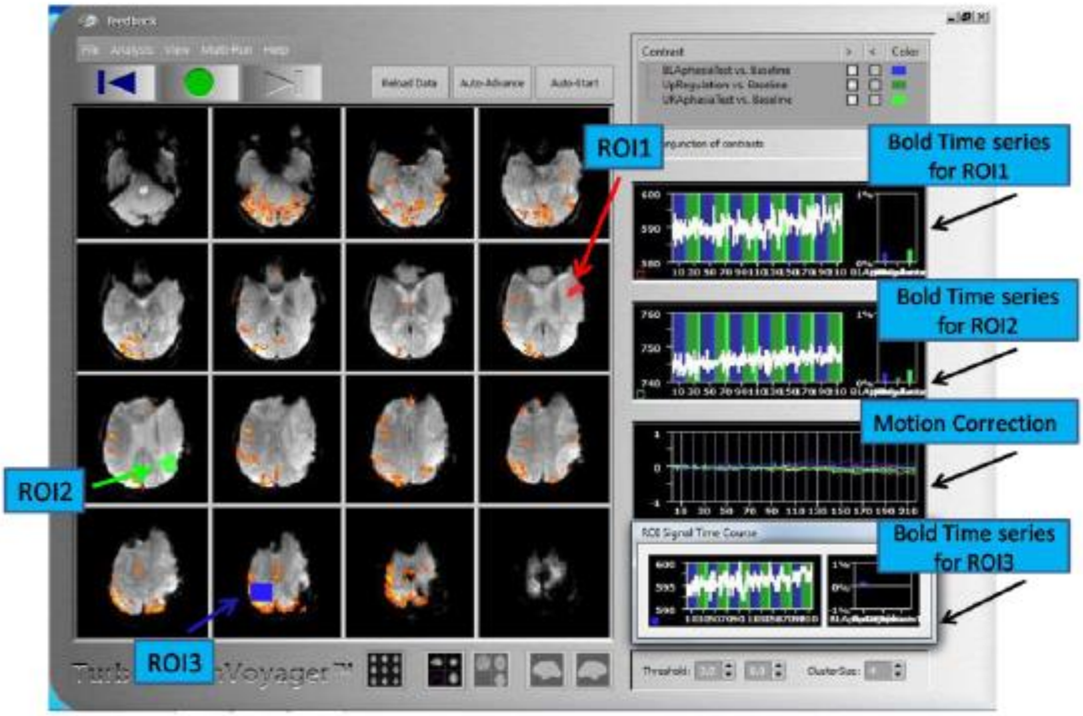


Fig.6: Turbo Brain Voyager window during RT-fMRI run

3.3. NEUROFEEDBACK

The BOLD feedback was displayed, visually as a bar graph meter during the up-regulation tasks. The baseline level zero was presented as 10 levels in blue colour. An enhancement in the BOLD feedback was shown as a proportionate increase in levels in red. If the BOLD activation fell below the baseline, the blue levels were accordingly removed. The feedback visuals were produced with the help of Presentation software. The subjects being scanned were advised to raise the bars in the meter by covert speech exercises.

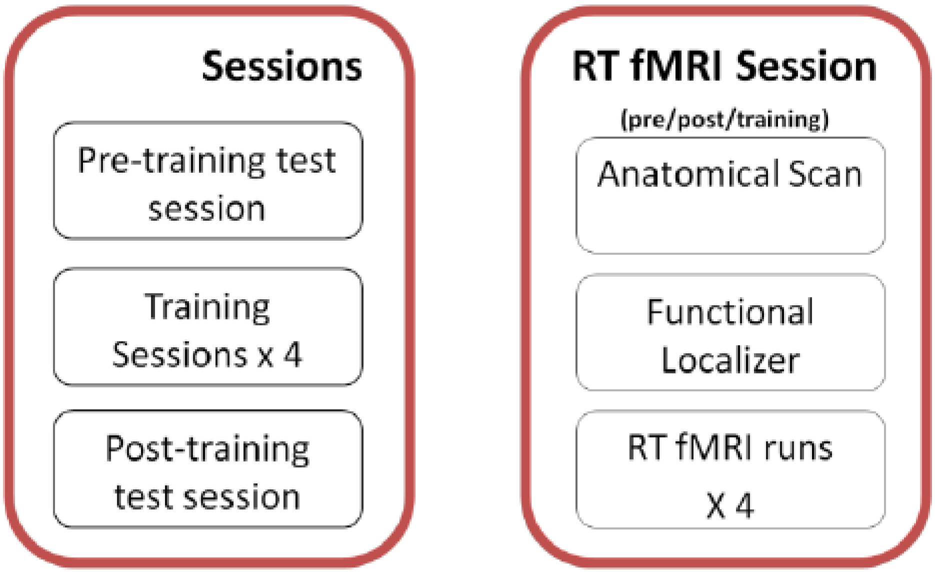


Fig.7: Scans in a single session

3.4. REAL-TIME fMRI NEUROFEEDBACK SESSIONS

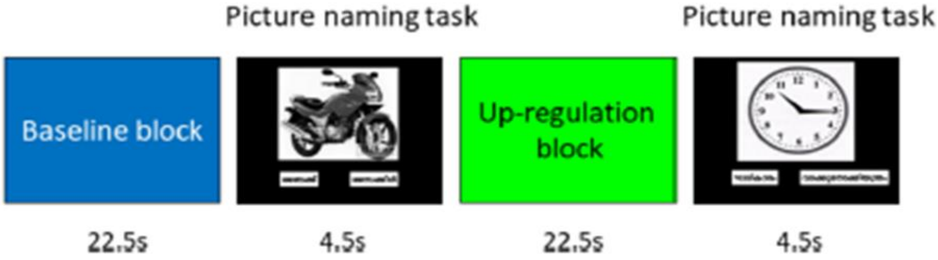
The RT-fMRI-based neurofeedback training was conducted in four sessions scheduled over a few weeks. The training was performed only for the test patients, and the normal subjects whereas for control patients only test sessions were performed for two to four weeks. Every session spanned approximately one hour. The initial step was to delineate the ROIs associated with language using a functional localizer task. These ROIs were used for RT-fMRI-based neurofeedback sessions.

The first session was a pre-training test session which included a picture naming task (test) after every baseline block and up-regulation block. The next four sessions were training sessions that did not have any test task and consisted of alternating baseline and up-regulation blocks. In the baseline block, the patient was provided the blue background as a cue to rest in the scanner. The BOLD feedback values were not presented to the patient in the baseline block. In the upregulation task block in which the feedback was presented and the patient had to try to increase the feedback levels displayed by exercising the patient's language areas, the patient was provided a green background as a cue to exercise language function and activate the language areas. This was followed by a post-training test session, which was similar to the pre-training test session. The participants were asked to covertly name the object displayed and make a selection of the correct name from two given choices by pressing the left or right button.

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
TEST	Pre Test Session A B C	Training Session 1 A B C	Training Session 2 A B C	Training Session 3 A B C	Training Session 4 A B C	Post Test Session A B C
CONTROL	Pre Test Session A B C	Post Test Session A B C				
NORMAL	Pre Test Session A B C	Training Session 1 A B C	Training Session 2 A B C	Training Session 3 A B C	Training Session 4 A B C	Post Test Session A B C

A: Anatomical Scan (6 minutes)
B: Functional Localizer (3 minutes)
C: RT-fMRI Runs 1,2,3 & 4. (30 minutes)

(a) Neurofeedback sessions underwent by test, control, and normal subjects
(Each session comprised of an anatomical scan (A), a functional localizer (B), and four RT-fMRI neurofeedback runs (C))



(b) Schematic of a Test Session Run (pre and post)

Fig. 8: Neurofeedback Training and Test Sessions

(Source: Sreedharan et al., 2019)

The activity generated in the brain in the subjects, while reacting to the coloured backgrounds in baseline and upregulation blocks, was avoided from being captured in the acquired fMRI images with the help of CONN software in order to get information on functional connectivity among those brain regions only which are activated during the recovery of language function.

3.5. ANALYSIS OF DATA

The fMRI data were analysed in SPM8 software to check the level of functional connectivity in the language regions and the adjoining areas. The pre-processing involved realignment, coregistration, smoothing, and normalization. The up-regulation blocks for all four runs in every session were selected to produce the contrast. Then a second level t-test was performed on the contrast maps to identify areas where prominent up-regulation activity was detected for all six sessions. An ROI level estimation of the contrast value, its statistical implication, and percentage signal change during up-regulation blocks (UR) was worked out in SPM. Likewise, perilesional ROIs were also examined for patient groups in both the Broca's and Wernicke's areas. The perilesional ROIs were also demarcated with the help of AAL (Automated Anatomical Labeling) space. The presentation of the significance of the statistic t-tests was presented as p-value<0.05(*), p-value<0.01(**), p-value<0.001(***), p-value<0.0001 in the connectivity matrices.

Data from the task were studied on the basis of the score and the time of response in the naming task. The examination of results was done after each baseline block and up-regulation block in each test session and between the pre-training test session and post-training test session. The effect of the training on the language performance of the subjects was measured by a WAB test before and after the RT-fMRI sessions.

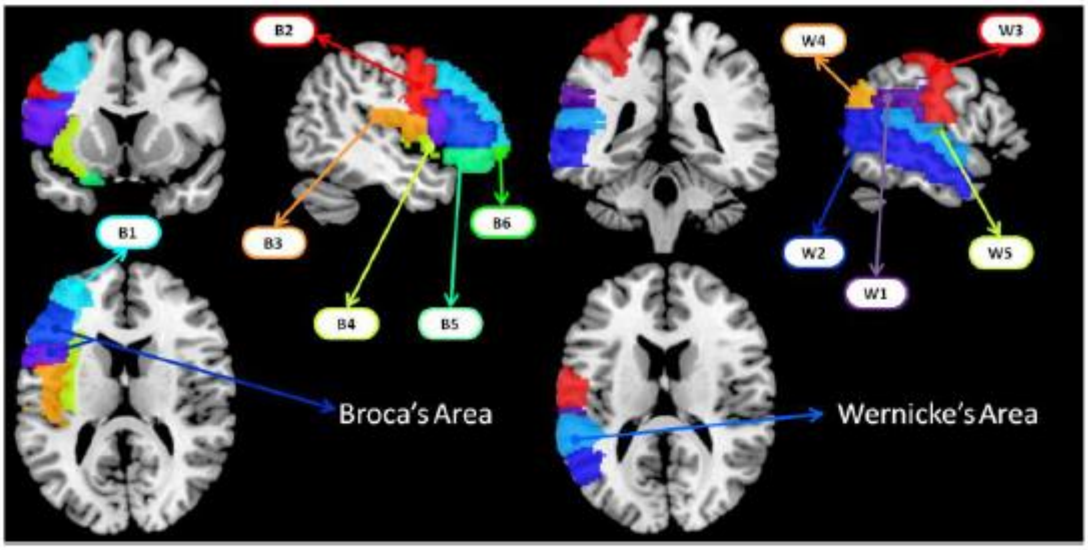


Fig.9: ROIs in language area and perilesional ROIs

(Source: Sreedharan et al., 2019)

Perilesional ROIs of Broca's area in the Left Hemisphere		
B1	Frontal Mid L	Middle Frontal gyrus
B2	Precentral L	Precentral gyrus
B3	Rolandic Oper L	Rolandic operculum
B4	Insula L	Insula
B5	Frontal Inf Orb L	Inferior orbital gyrus
B6	Frontal Mid Orb L	Middle orbital gyrus
Perilesional ROIs of Wernicke's area in the Left Hemisphere		
W1	SupraMarginal L	Supramarginal gyrus
W2	Temporal Mid L	Mid Temporal gyrus
W3	Postcentral L	Postcentral gyrus
W4	Angular L	Angular gyrus
W5	Heschl L	Heschl's gyrus

Table 5: Perilesional areas of language regions

3.6. FUNCTIONAL CONNECTIVITY

Analysis of functional connectivity among the ROIs was done with the use of CONN toolbox. All the patient groups were analysed under one project with all the twelve subjects assembled properly. Normal and test participants had 24 conn-sessions (6 sessions x 4runs/session) and control subjects had 8 conn-sessions (2 sessions x 4 runs/session). The sessions were classified as sessions 1 to 6, the rest condition spanning all conn-sessions, and baseline (BL), up-regulation (UR), post-baseline test, and post-up-regulation test conditions as defined in the SPM general linear model. The anatomical images were co-registered and segmented into grey matter, white matter, and Cerebrospinal fluid. CONN made use of the motion correction regressors from SPM and a CompCor algorithm to suppress the effects of these factors, and then the BOLD signal was used for estimating the connectivity correlation.

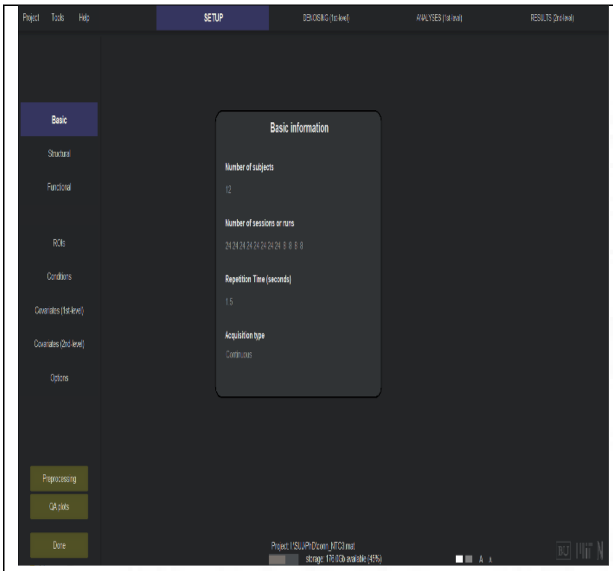
The ROIs chosen for the experiment were the Broca's and the Wernicke's areas and their right homologous regions, and eleven other ROIs around the Broca's and the Wernicke's areas (restricted to each patient's active region in an SPM t-test of p-value < 0.01 in the up-regulation task). Additionally, 50 ROIs from the AAL (Tzourio-Mazoyer et al., 2002) space were also used which were grouped in several modules.

The first-level ROI to ROI analysis was performed for all the 65 ROIs as seeds. The bandpass filter setting was in the frequency range of 0.008 Hz to 0.09 Hz. In the second-level analysis, Analysis of Variance (ANOVA) was performed with the inter-subject group and inter-conditional contrasts. The measure for functional connectivity among the ROIs used in the experiment is the Pearson's correlation coefficient for two ROIs' time series x and y as shown below (Equation 2):

$$corr(x,y) = x^t y/\{||x||.||y||\} \qquad (2)$$

Analysis of the connectivity matrix which is acquired for the normal group is done for modularity and ROIs are split into four modules in the left hemisphere. The first module that is, the frontotemporal language network is further divided based on anatomical position.

The modules were restricted to the left hemisphere and six other homologous modules were generated for the right hemisphere. The 12 modules so generated were examined for modifications in functional connectivity.



Basic information

Number of subjects
12

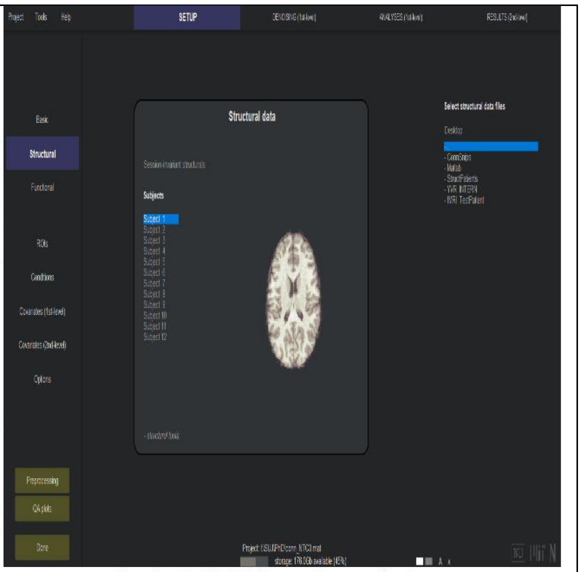
Number of sessions or runs
24 24 24 24 24 24 24 24 8 8 8 8

Repetition Time (seconds)
1.5

Acquisition type
Continuous

Project: I:\SU\PhD\conn_NTC3.mat
storage: 176.0Gb available (45%)

(a) Basics



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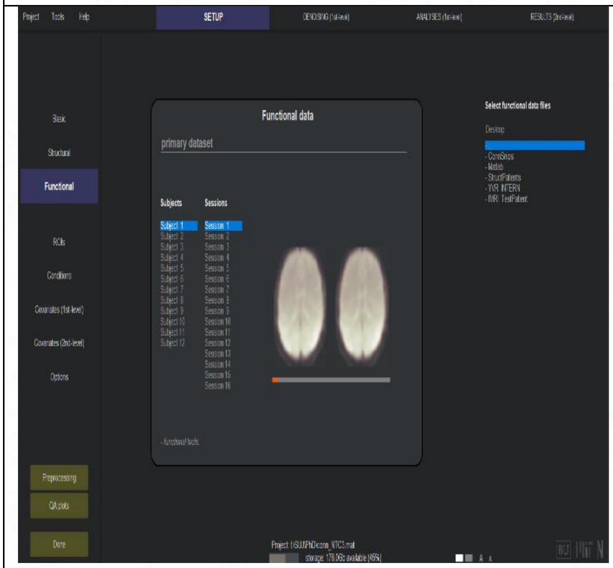
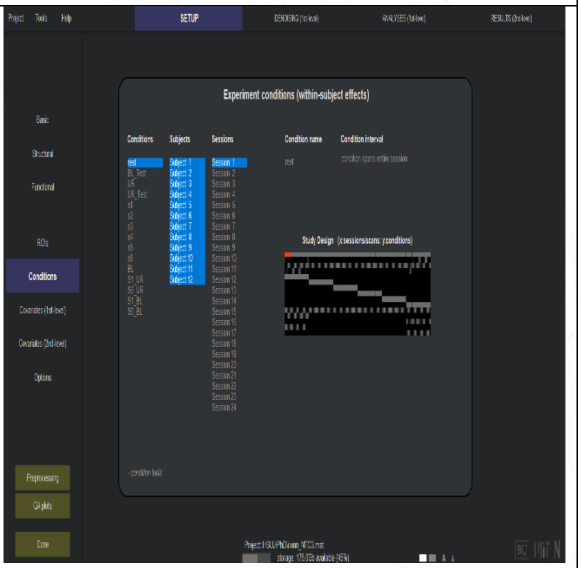
storage: 176.0Gb available (45%)

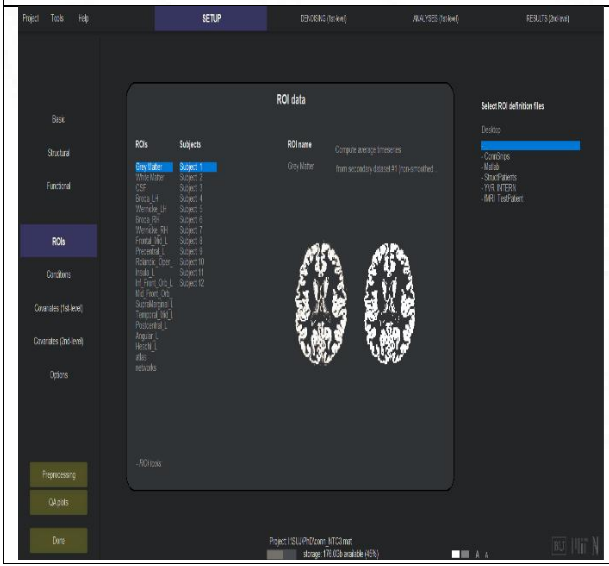
(b) Structural

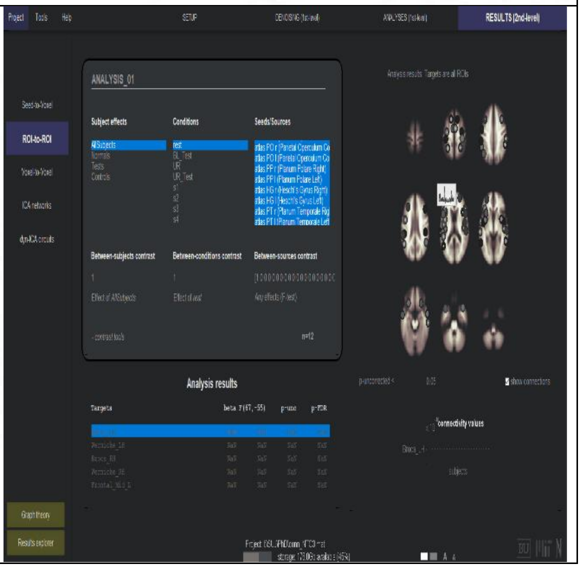


(c) Functional



(d) Conditions





(e) ROI	(f) Results (2nd level)

Fig.10: Tabs of operations performed on CONN toolbox

Left Hemispheric ROIs

FL	Frontal Language	Broca_L*, FrontalMid_L*, SFG, MFG, IFG triangularis, IFG operculum, Frontal operculum (FO) [FL-1 to FL-7]
TL	Temporal Language	Wernicke_L*, TemporalMid_L*, Angular_L*, pSTG, pMTG, toMTG, pSMG, Angular gyrus (AG) [TL-1 to TL-8]
CO	Central Opercular	RolandicOper_L*, Insula_L*, Hesch_L*, Insula, Central operculum (CO), Parietal operculum (PO), Planum polare (PP), Heschl's gyrus (HG), Planum temporale (PT) [CO-1 to CO-9]
FP	Frontal Polar	FrontalInfOrb_L*, FrontalMidOrb_L*, Frontal pole (FP), Frontal orbitalis (FO) [FP-1 to FP-4]
TP	Temporal Polar	Temporal pole (TP), aSTG, aMTG [TP-1 to TP-3]
SP	Supra Parietal	Precentral_L*, Supramarginal_L*, Postcentral_L*, Precentral gyrus (PreCG), Postcentral gyrus (PostCG), SPL, aSMG. [SP-1 to SP-7]

Right Hemispheric ROIs

rFL	Right Frontal Language	Broca_R*, SFG.r, MFG.r, IFGtri.r, IFGoper.r, FO.r [rFL-1 to rFL-6]
rTL	Right Temporal Language	Wernicke_R*, pSTG.r, pMTG.r, toMTG.r, pSMG.r, AG.r [rTL-1 to rTL-6]
rCO	Right Central Opercular	Insula_R, CO.r, PO.r, PP.r, HG.r, PT.r [rCO-1 to rCO-6]
rFP	Right Frontal Polar	right Frontal pole (FP.r), right Frontal orbitalis. [rFP-1 to rFP-2]
rTP	Right Temporal Polar	right Temporal pole, aSTG.r, aMTG.r [rTP-1 to rTP-3]
rSP	Right Supra Parietal	PreCG.r, PostCG.r, SPL.r, aSMG.r [rSP-1 to rSP-4]

Table 6: Grouping of ROIs into modules

Intramodular connectivity was obtained for each of the 12 modules, in the form of connectivity matrices, displaying the functional connectivity of each ROI present in a module, with every other ROI present in the same module. The connectivity matrices were plotted using BrainNet for rest, baseline (BL), and up-regulation (UR) conditions. Intramodular functional connectivity of the test, normal and control groups were estimated under these conditions and for final sessions (2nd session for controls and 6th session for test and normal groups).

Inter-group comparisons that were performed are:

(A) Normal group > Test group during first session,

(B) Normal group> Test group during rest condition,

(C) Test group> Control group in final session over first session,

(D) Test group> Control group in final session over first session under baseline condition,

(E) Test group> Control group, in final session over first session, Up-regulation> Baseline.

Intra-group comparisons that were performed are:

(F) Up-regulation > Baseline for Test group,

(G) Second half of sessions > First half for Test group,

(H) Up-regulation > Baseline for Normal group,

(I) Second half of sessions > First half for Normal group,

(J) Final Session > First Session for Test group during baseline condition,

(K) Final session> First session for Normal group,

(L) Final Session > First Session for Test group during up-regulation condition,

(M) Final session > First session, and up-regulation> baseline for Test group,

(N) Final Session > First Session for Test group.

For intra-group comparisons, the intra-modular functional connectivity was statistically tested against the alternate hypothesis of a non-zero mean with a p-value of 0.05 using a t-test with unknown mean and unknown variance, on MATLAB. For inter-group comparison, the statistical test used was the t-test for two groups with unknown means and unknown but equal variances against the alternate hypothesis of different means with a p-value of 0.05.

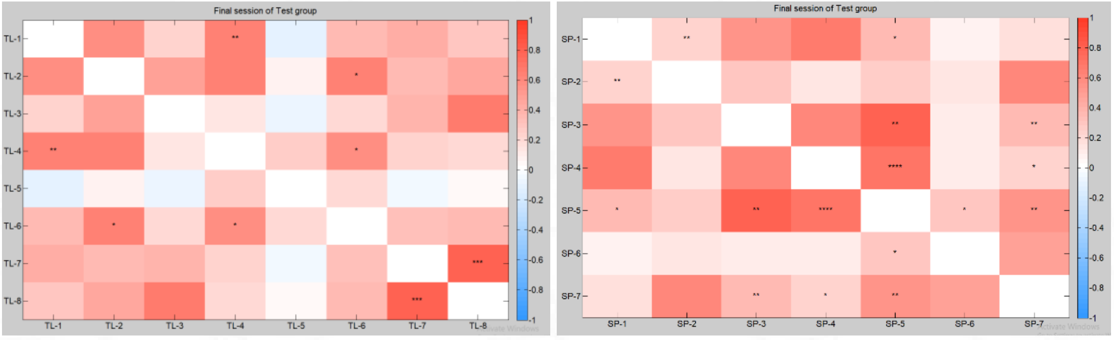
CHAPTER 4

RESULTS

4.1. FUNCTIONAL CONNECTIVITY IN THE THREE GROUPS OF SUBJECTS

Analysis of functional connectivity between ROIs present within various modules, under varying conditions, has been done in this section.

Final session of Test group:



(a) Module Temporal Language (b) Module Supra Parietal

Fig.11: Intramodular Connectivity Matrices of Final Session of Test group (p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

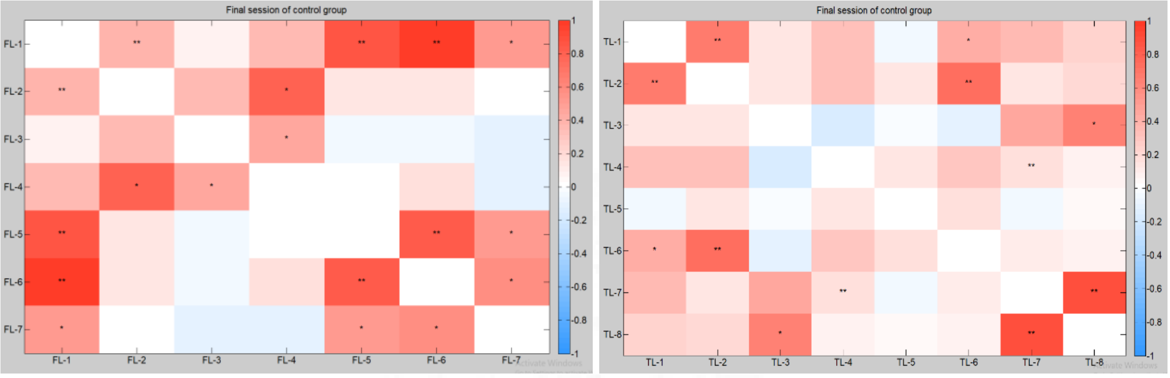
Intra-modular connectivity matrix of Temporal Language (TL) in the left hemisphere of test patients, obtained for the final, that is, 6th session shows that strong FC exists between ROIs TL-7, and TL-8 (Refer to Table 6 for names of the ROIs) (Figure 11 (a)).

In Supra Parietal module in the left hemisphere, the strength of FC is good between ROIs SP-4, and SP-5 (Figure 11 (b)).

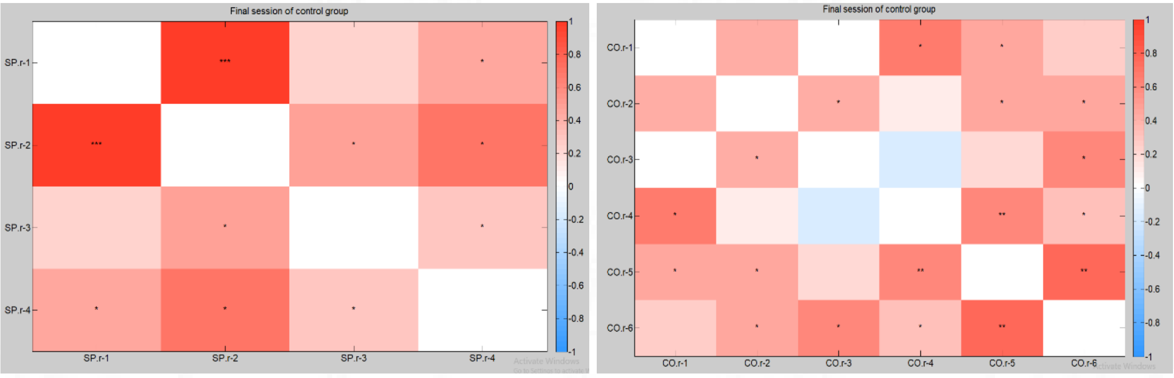
Final session of control group:

For the control group, analysis of the final session, that is the 2nd session, shows that ROIs FL-2, and FL-4 have strong FC in Frontal Language module in the left hemisphere (Figure 12 (a)).

Similarly, in Temporal Language module, ROIs TL-7, and TL-8 have good strength of FC (Figure 12 (b)).



(a) Module Frontal Language (b) Module Temporal Language



(c) Module Supra Parietal (right) (d)Module Central Opercular (right)

Fig. 12: Intramodular Connectivity Matrices of Final Session of Control group

($p<0.05$(*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****); The colour bar indicates the strength between pairs of ROIs.)

In the right hemisphere, ROIs SP.r-1, and SP.r-2, in the Supra Parietal (right) module have good FC (Figure 12 (c)).

ROIs CO.r-5, and CO.r-6, in the Central Opercular (right) module, in the right hemisphere have strong FC between them (Figure 12 (d)).

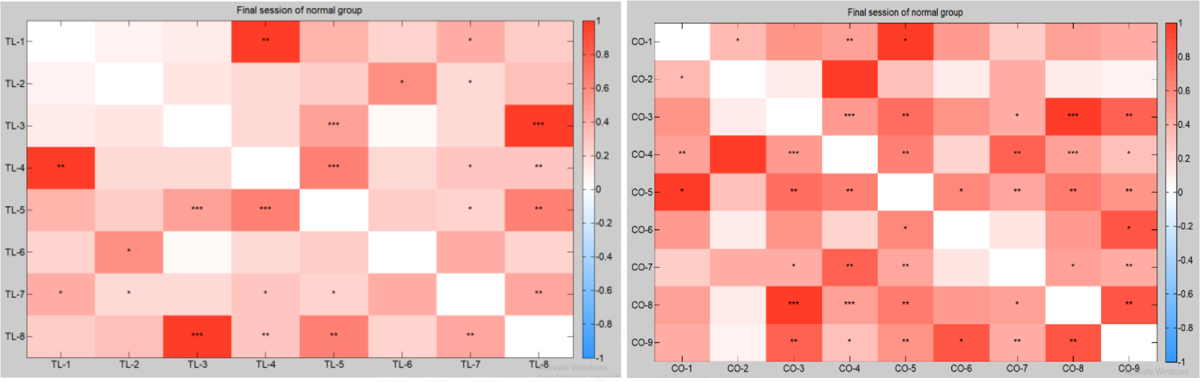
Final session of normal group:

In the final session of the normal group, that is, the 6th session, TL-3, and TL-8 of the Temporal Language module show strong FC (Figure 13 (a)).

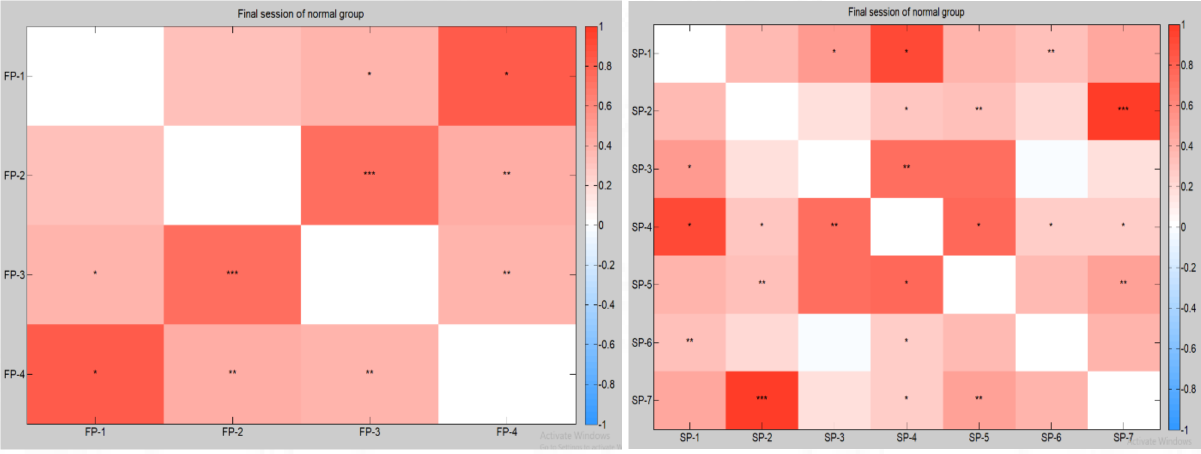
In the Central Opercular module, CO-3, and CO-8, show significant FC (Figure 13 (b)).

FP-2, and FP-3 of the Frontal Polar module have strong FC (Figure 13 (c)).

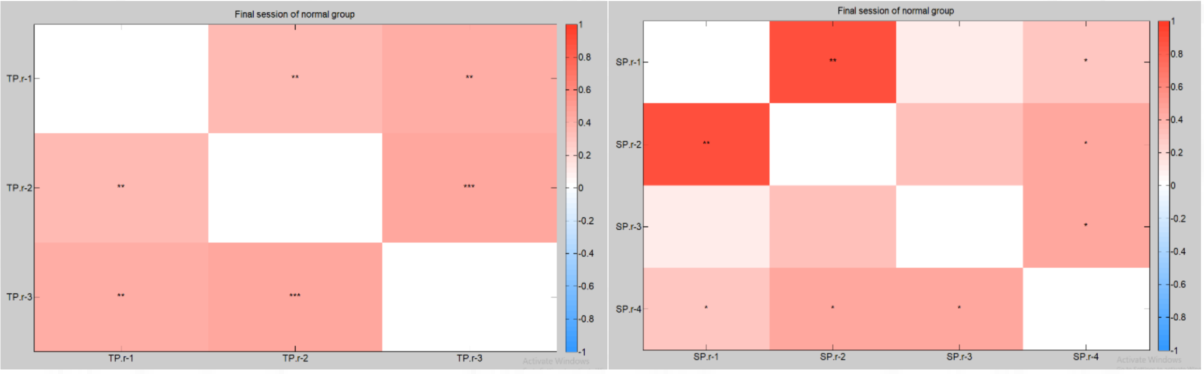
In the Supra Parietal module, SP-2, and SP-7 show significant FC (Figure 13 (d)).



(a) Module Temporal Language (b) Module Central Opercular



(c) Module Frontal Polar (d) Module Supra Parietal



(e) Module Temporal Polar (right) (f) Module Supra Parietal (right)

Fig. 13: Intramodular Connectivity Matrices of Final Session of Normal group

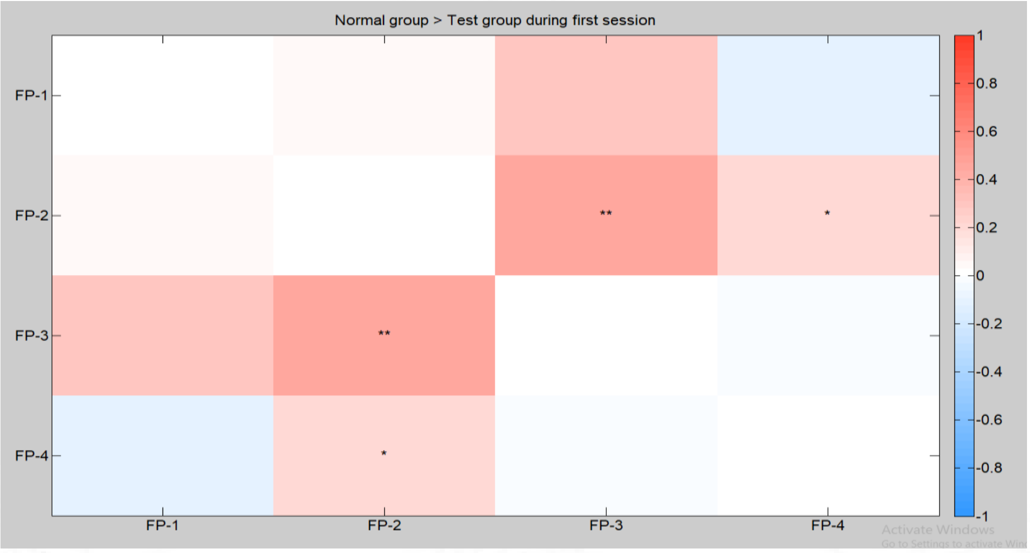
(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

In the right hemisphere, ROIs TP.r-2, and TP.r-3, in the Temporal Polar (right) module, have strong FC (Figure 13 (e)).

Likewise, ROIs SP.r-1, and SP.r-2, in the Supra Parietal (right), show strong FC (Figure 13 (f)).

Inter-group comparisons:

(A) Normal group > Test group during first session

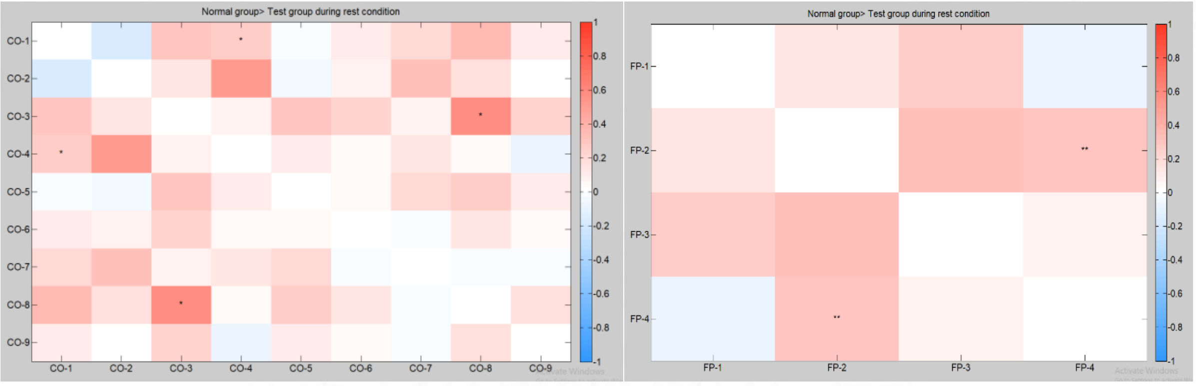


(a) Module Frontal Polar

Fig. 14: Intramodular Connectivity Matrix: Normal group > Test group during first session (A)
(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

In the intergroup comparison between normal and test subjects, during the first session (Normal>Test), in the Frontal Polar module, in the left hemisphere, ROIs FP-2, and FP-3 have strong FC between them (Figure 14 (a)).

(B) Normal group> Test group during rest condition



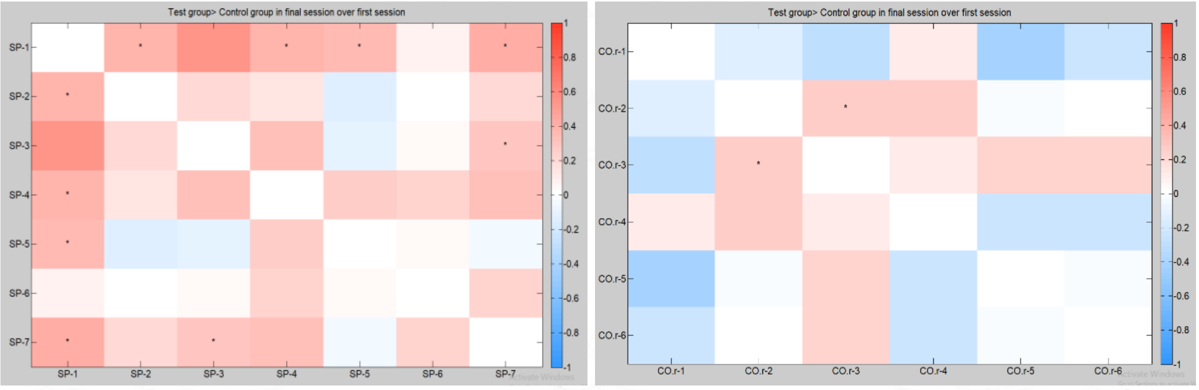
(a) Module Central Opercular (b) Module Frontal Polar

Fig. 15: Intramodular Connectivity Matrix: Normal group> Test group during rest condition (B)
(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

On comparing the normal and the test groups were compared under rest condition, it has been observed that in the central opercular module, ROIs CO-3, and CO-8 have strong FC (Figure 15 (a)).

Again, in the Frontal Polar module, in the left hemisphere, ROIs FP-2, and FP-4, have strong FC (Figure 15 (b)).

(C) Test group> Control group in final session over first session



(a) Module Supra Parietal (b) Module Central Opercular (right)

Fig. 16: Intramodular Connectivity Matrix: Test group> Control group in final session over first session (C)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

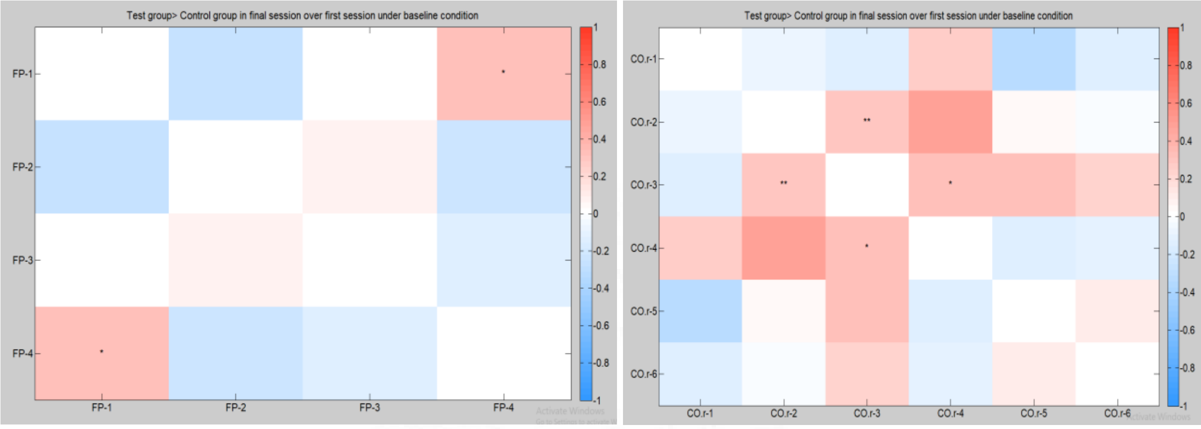
In the inter-group comparison between the test and the control group, it has been observed that in the Supra Parietal module in the left hemisphere, ROIs SP-1, and SP-7 have strong FC between them (Figure 16 (a)).

In the right hemisphere, ROIs CO.r-2, and CO.r-3 in the Central Opercular (right) module, have good strength of FC (Figure 16 (b)).

(D) Test group> Control group in final session over first session under baseline condition

On comparing the test, and control groups, in final session over first session, under baseline condition, it has been found that, ROIs FP-1, and FP-4, present in the Frontal Polar module, in the left hemisphere, have good FC (Figure 17 (a)).

In the right hemisphere, in the Central Opercular (right) module, ROIs CO.r-2, and CO.r-3 show strong FC (Figure 17 (b)).



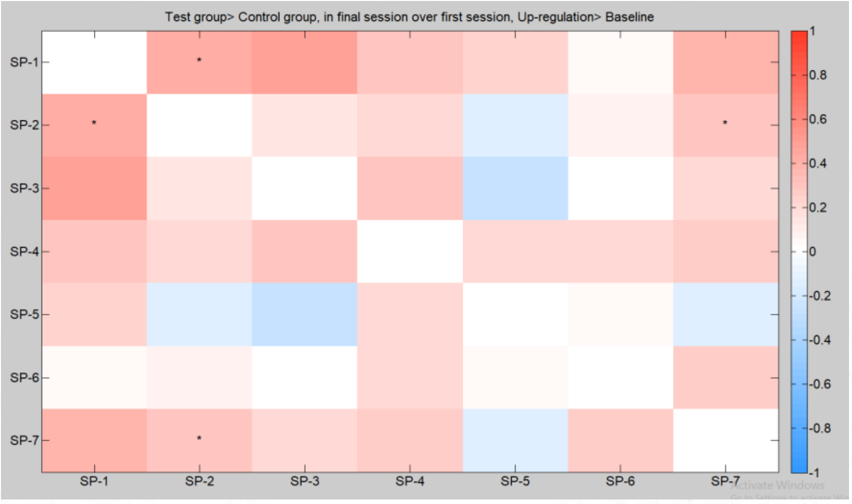
(a) Module Frontal Polar (b) Module Central Opercular (right)

Fig. 17: Intramodular Connectivity Matrix: Test group> Control group in final session over first session under baseline condition (D)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

(E) Test group> Control group, in final session over first session, Up-regulation> Baseline

In the intra-group comparison between the test, and control groups, in final session over first session, under up-regulation condition over baseline condition, it has been found that in the left hemisphere, ROIs SP-1, and SP-2 present in the Supra Parietal module show good FC (Figure 18 (a)).



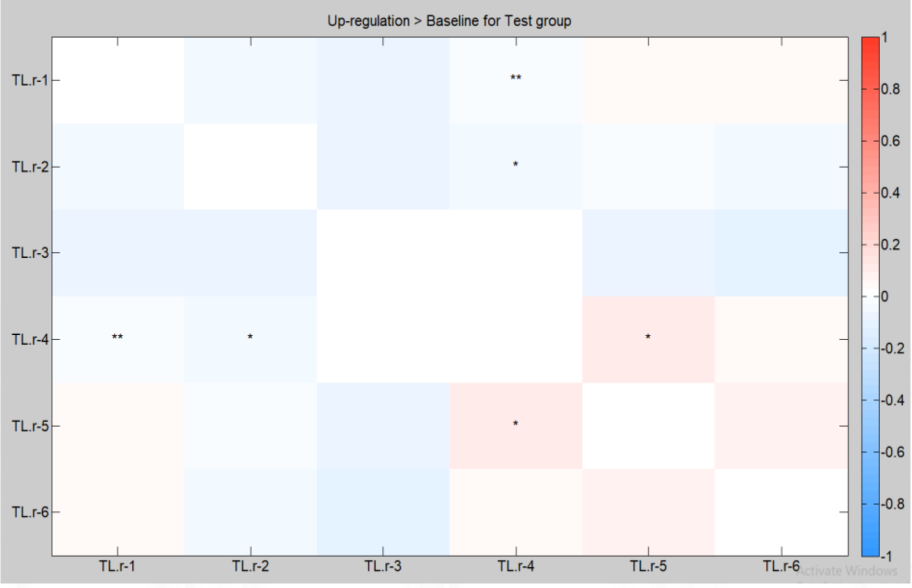
(a) Module Supra Parietal

Fig. 18: Intramodular Connectivity Matrix: Test group> Control group, in final session over first session, Up-regulation> Baseline (E)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

Intra-group comparisons:

(F)Up-regulation > Baseline for Test group



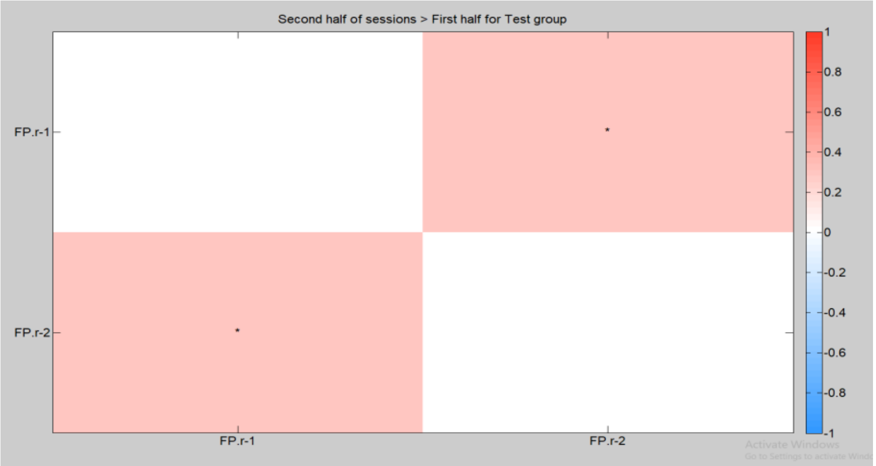
(a) Module Temporal Language (right)

Fig. 19: Intramodular Connectivity Matrix: Up-regulation > Baseline for Test group (F)
(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

In the intra-group comparison of up-regulation condition over baseline condition, involving the test group, it has been found that in the right hemisphere, ROIs TL.r-4, and TL.r-5, present in the Temporal Language (right) module show strong FC (Figure 19 (a)).

(G) Second half of sessions > First half for Test group

On comparing the second half of sessions over the first half of sessions in the test group, it has been found that in the right hemisphere, ROIs FP.r-1, and FP.r-2, present in the Frontal Polar (right) module, show strong FC (Figure 20 (a)).



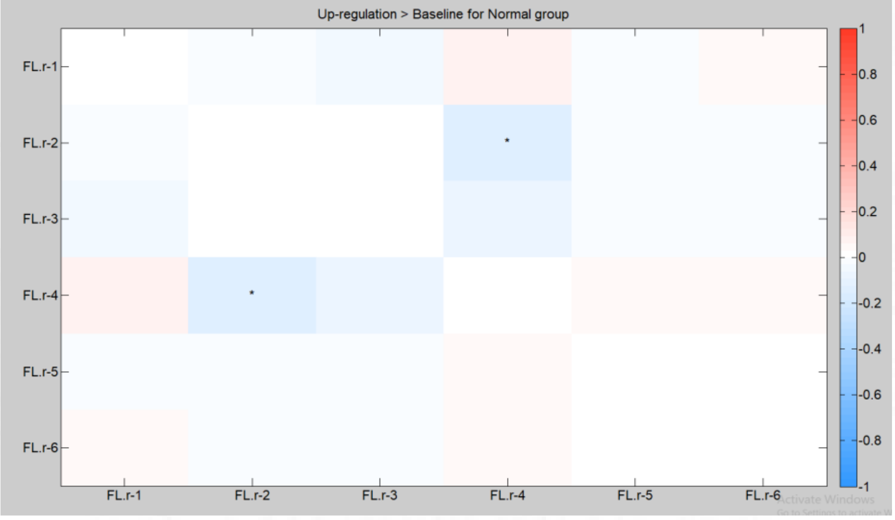
(a) Module Frontal Polar (right)

Fig. 20: Intramodular Connectivity Matrix: Second half of sessions > First half for Test group (G)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

(H) Up-regulation > Baseline for Normal group

In the intra-group comparison of up-regulation condition over baseline condition in the normal group, it has been observed that, in the right hemisphere, ROIs FL.r-1, and FL.r-4, present in the Frontal Language (right) module, have good FC, when compared to other ROIs (Figure 21 (a)).

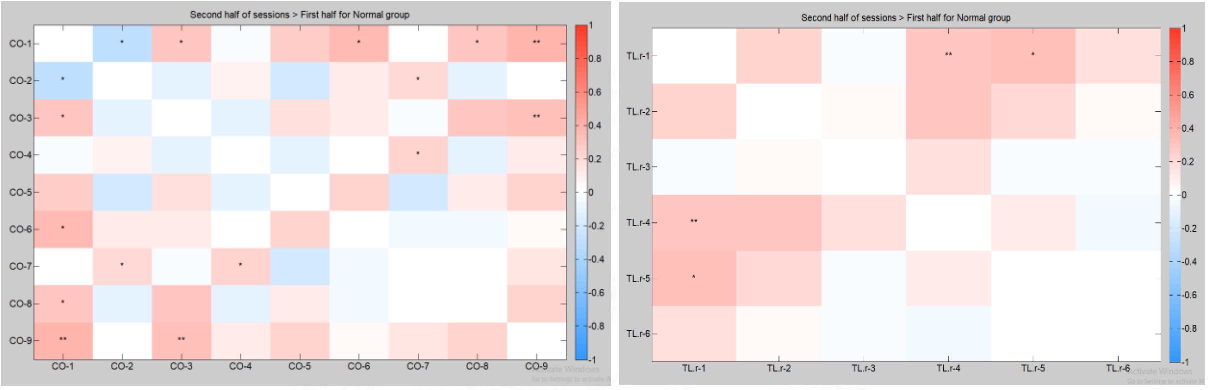


(a) Module Frontal Language (right)

Fig. 21: Intramodular Connectivity Matrix: Up-regulation > Baseline for Normal group (H)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar the strength between pairs of ROIs.)

(I) Second half of sessions > First half for Normal group



(a) Module Central Opercular (b) Module Temporal Language (right)

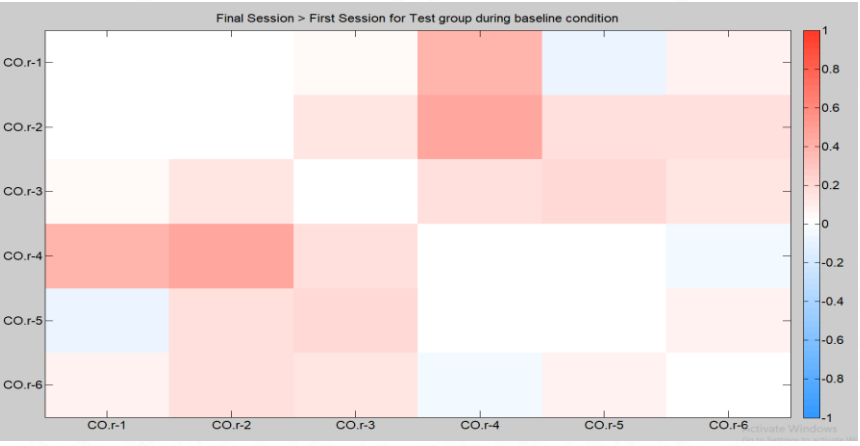
Fig. 22: Intramodular Connectivity Matrix: Second half of sessions > First half for Normal group (I)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar the strength between pairs of ROIs.)

On comparing the second half of sessions over the first half of sessions for the normal group, it has been found that ROIs CO-1, and CO-9, present in the Central Opercular module in the left hemisphere, have good strength of FC (Figure 22 (a)).

Likewise, ROIs TL.r-1, and TL.r-4 in the right hemispheric module of Temporal Language (right) have good strength of FC (Figure 22 (b)).

(J) Final Session > First Session for Test group during baseline condition



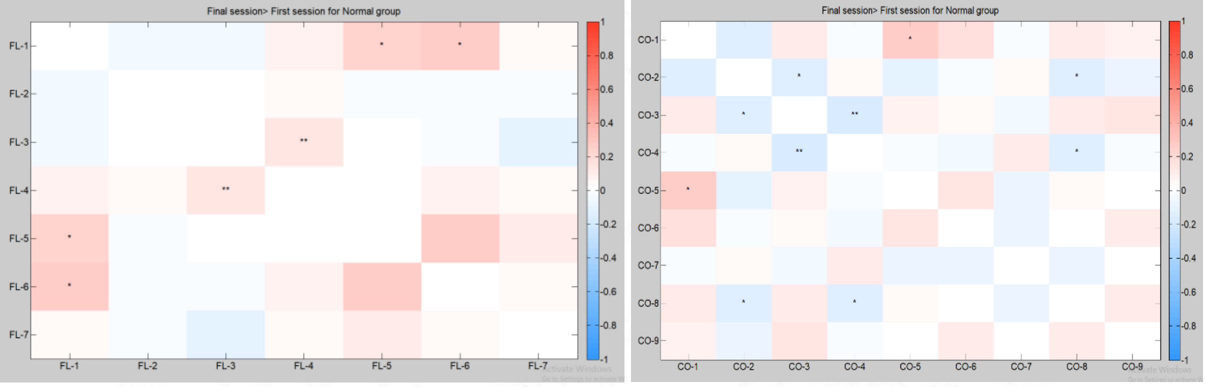
(a)Module Central Opercular (right)

Fig. 23: Intramodular Connectivity Matrix: Final Session > First Session for Test group during baseline condition (J)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar the strength between pairs of ROIs.)

On comparing the final session of the test group over it’s first session, under baseline condition, it has been found that ROIs CO.r-2, and CO.r-4, present in the right hemisphere in module Central Opercular (right), show good FC (Figure 23 (a)).

(K) Final session> First session for Normal group

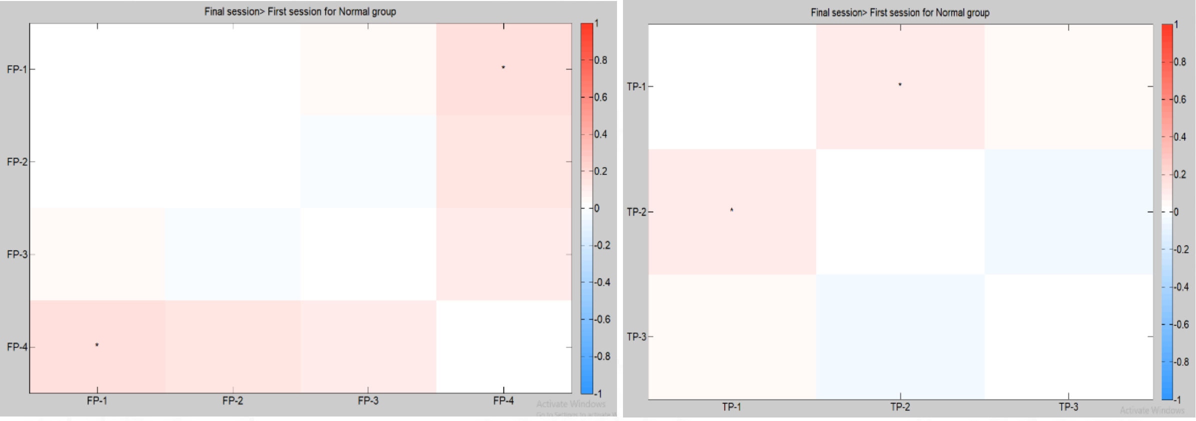


(a) Module Frontal Language

(b) Module Central Opercular

In the final session over first session comparison for the normal group, it has been found that there is strong FC between ROIs FL-1, and FL-4 in the Frontal Language module (Figure 24 (a)).

ROIs CO-1, and CO-5, of the Central Opercular module show good FC (Figure 24 (b)).



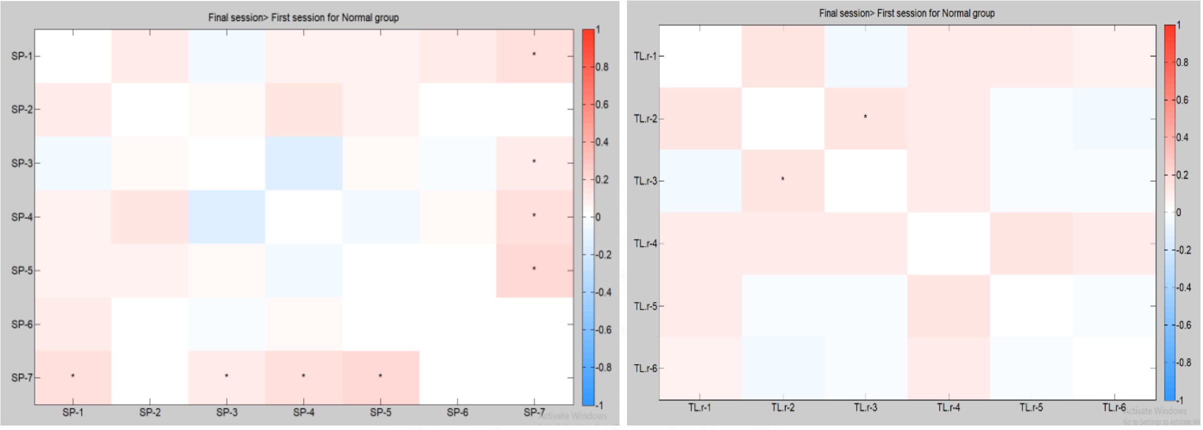
(c) Module Frontal Polar

(d) Module Temporal Polar

ROIs FP-1, and FP-4, of the Frontal Polar module, also show strong FC (Figure 24 (c)).

ROIs TP-1, and TP-2, in the left hemispheric Temporal Polar module show significant FC (Figure 24 (d)).

ROIs SP-1, and SP-5 have strong FC in Supra Parietal module (Figure 24 (e)).



(e) Module Supra Parietal (f) Module Temporal Language (right)

Fig. 24: Intramodular Connectivity Matrix: Final session> First session for Normal group (K)

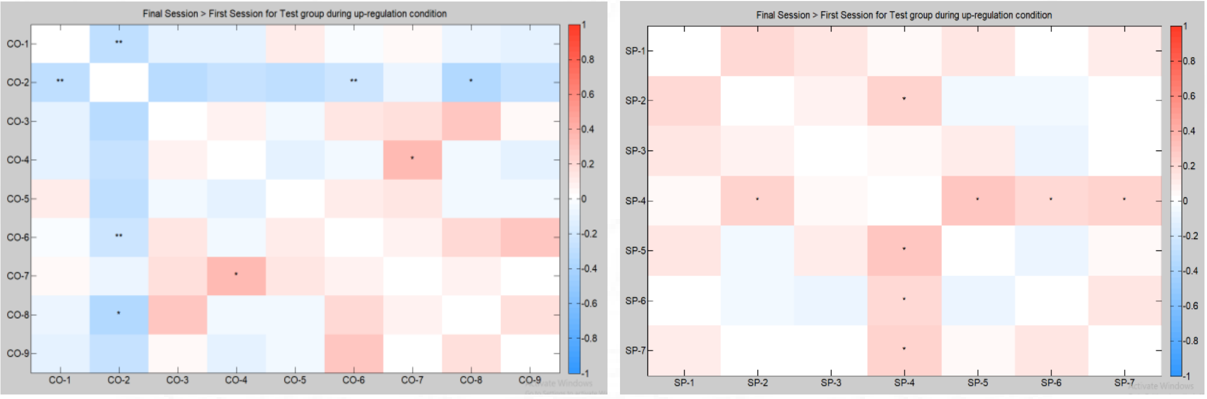
(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

In the right hemisphere, ROIs TL.r-2, and TL.r-3, in the Temporal Language (right) module, show good FC (Figure 24 (f)).

(L) Final Session > First Session for Test group during up-regulation condition

On comparing the final session over the first session, under up-regulation condition for test patients, it has been found that ROIs CO-4, and CO-7 in the Central Opercular module show strong FC (Figure 25 (a)).

Similarly, ROI SP-4 has strong FC with SP-5, and SP-7 in Supra Parietal module (Figure 25 (b))

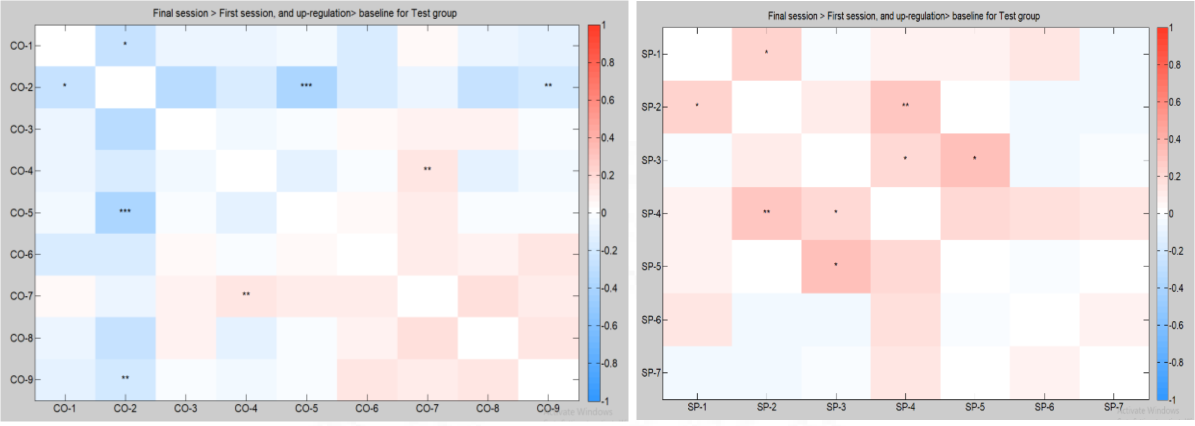


(a)Module Central Opercular (b) Module Supra Parietal

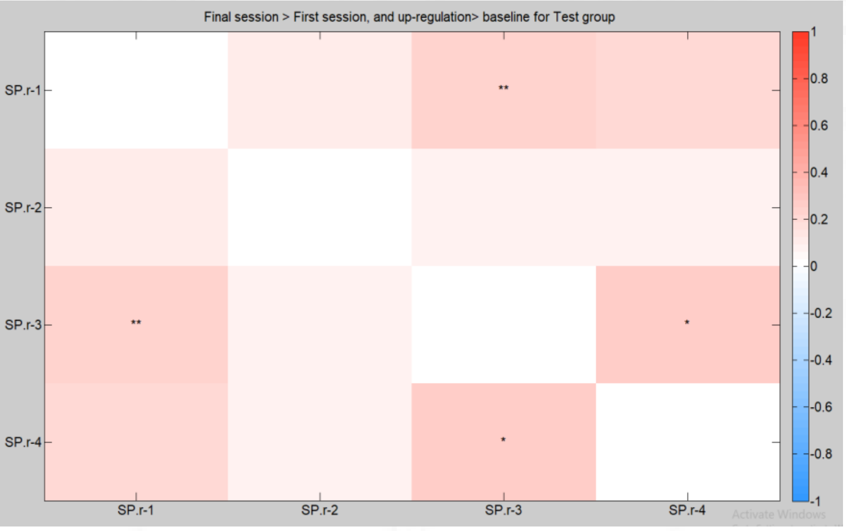
Fig. 25: Intramodular Connectivity Matrix: Final session > First session, and up-regulation> baseline for Test group (L)

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

(M) Final session > First session, and up-regulation> baseline for Test group



(a)Module Central Opercular (b) Module Supra Parietal



(c)Module Supra Parietal (right)

Fig. 26: Intramodular Connectivity Matrix: Final session > First session, and up-regulation> baseline for Test group (M)

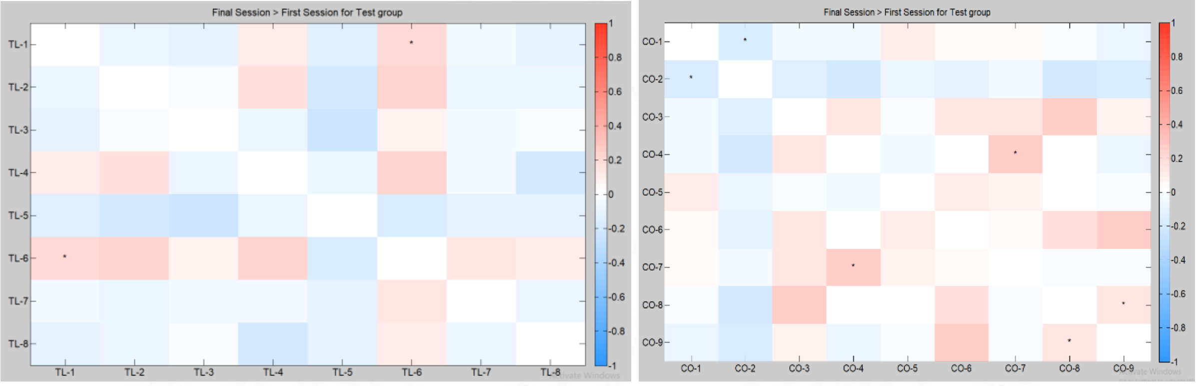
($p<0.05$(*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****); The colour bar the strength between pairs of ROIs.)

In the comparison of the final session over the first session, and up-regulation condition over baseline condition for test patients, it has been found that, in Central Opercular module, ROIs CO-4, and CO-7 have good FC (Figure 26 (a)).

FC between ROIs SP-2, and SP-4, and that between SP-3, and SP-5, is strong (Figure 26 (b)).

ROI SP.r-3 has good FC with ROIs SP.r-1, and SP.r-4, in the right hemispheric Supra Parietal module (Figure 26 (c)).

(N) Final Session > First Session for Test group



(a) Module Temporal Language (b) Module Central Opercular

Fig. 27: Intramodular Connectivity Matrix: Final Session > First Session for Test group (N)

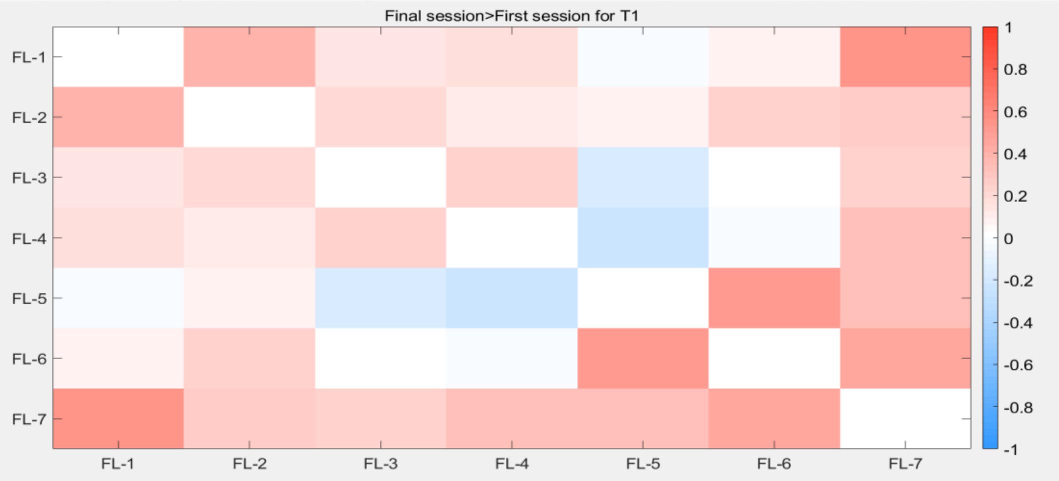
(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

On comparing the final session over the first session of test patients, it has been found that ROIs TL-1, and TL-6, have good FC in Temporal Language module (Figure 27 (a)).

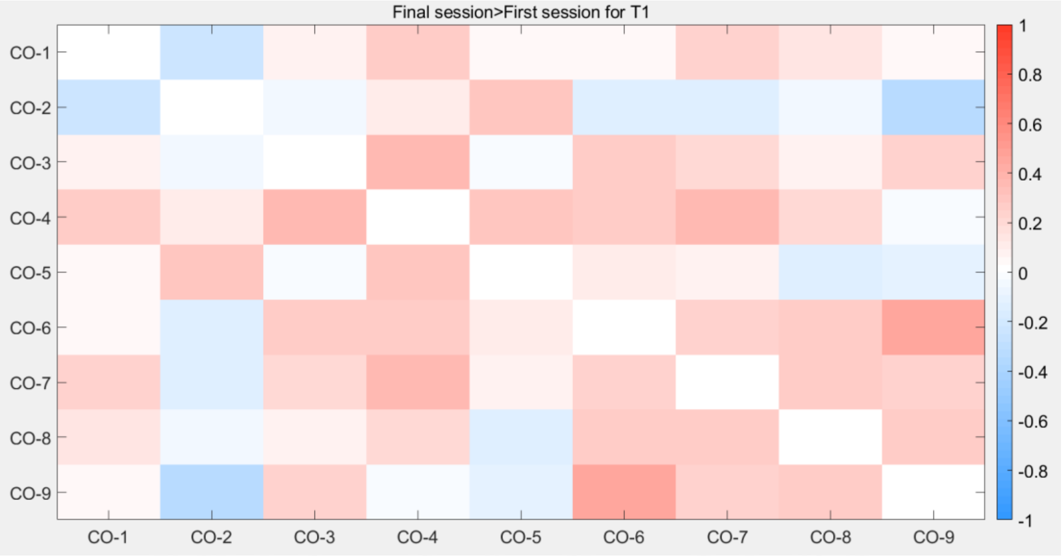
ROIs CO-4, and CO-7 in Central Opercular module have significant FC (Figure 27 (b)).

4.2. FUNCTIONAL CONNECTIVITY IN INDIVIDUAL TEST PATIENTS

Analysis of the 6th session over the first session for individual test patients has been done.



(a) Module Frontal Language



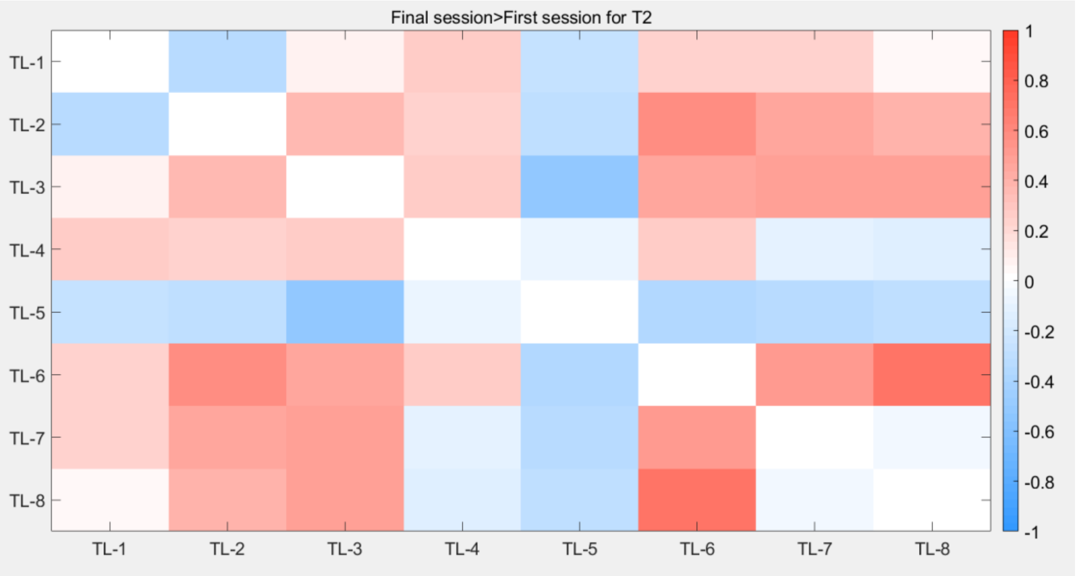
(b) Module Central Opercular

In the first test patient (T1), ROIs in the frontal language module, the central opercular module, and the right hemispheric central opercular (right) module, are found to have strong functional connectivity among themselves. ROI FL-1 has good connectivity with ROIs FL-2, and FL-7 (Figure 28 (a)). Similarly, ROI CO-4 has good FC with ROIs CO-3, and CO-7. ROIs CO-6, and CO-9 also have good FC (Figure 28 (b)). ROI CO.r-1 has good FC with ROIs CO.r-4, and CO.r-6. ROIs CO.r-2, and CO.r-4 have strong connectivity (Figure 28 (c)).

(c) Module Central Opercular (right)

Fig. 28: Intramodular Connectivity Matrix: Final Session > First Session for T1

($p<0.05$(*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****); The colour bar indicates the strength between pairs of ROIs.)

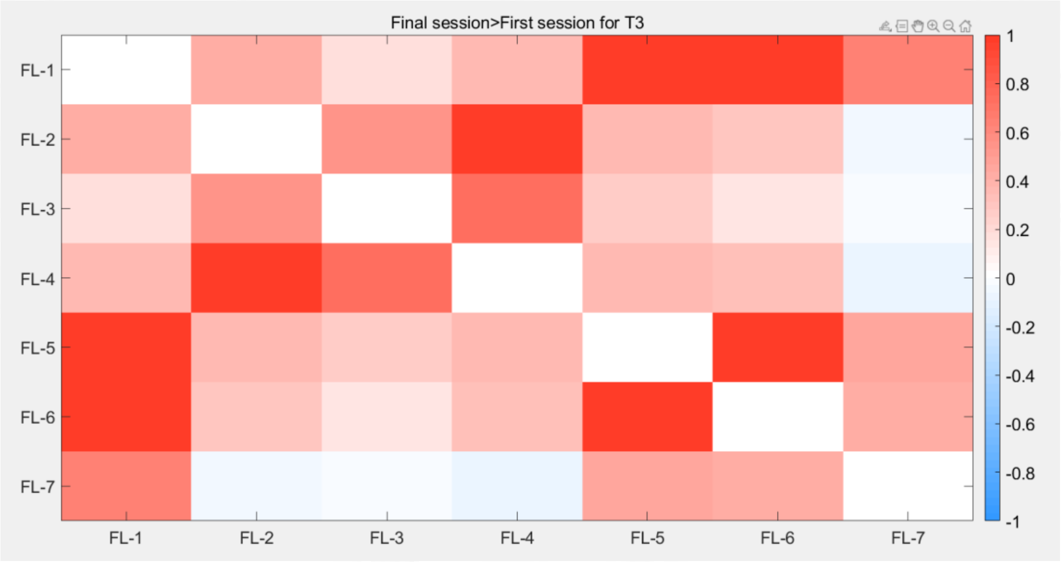


(a) Module Temporal Language

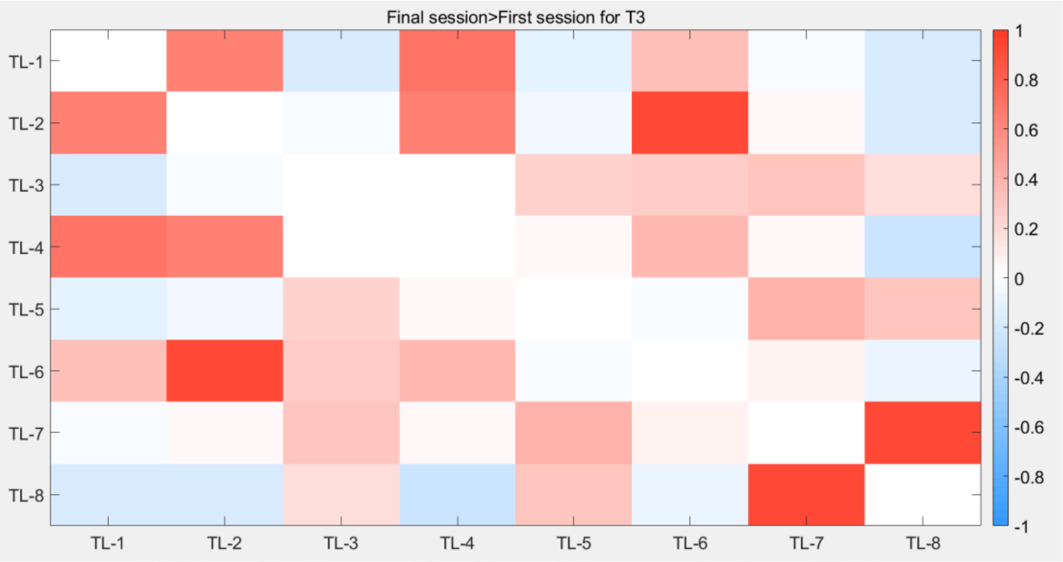
Fig. 29: Intramodular Connectivity Matrix: Final Session > First Session for T2

(p<0.05(*), p<0.01(**), p<0.001(***), p<0.0001(****); The colour bar indicates the strength between pairs of ROIs.)

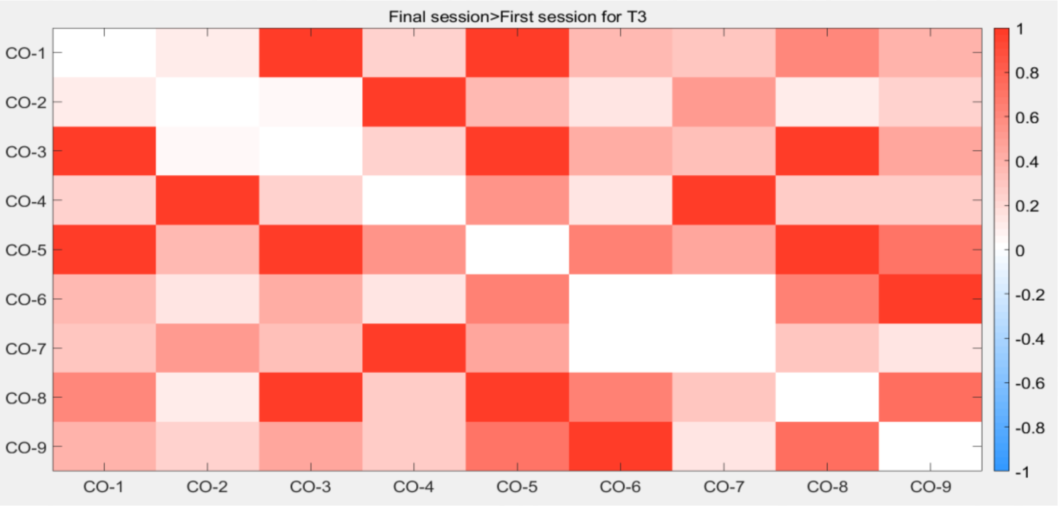
For the second patient (T2), ROIs in the temporal language module show strong FC among themselves. ROI TL-6 has good connectivity with ROIs TL-2, and TL-8 (Figure 29 (a)).



(a) Module Frontal Language

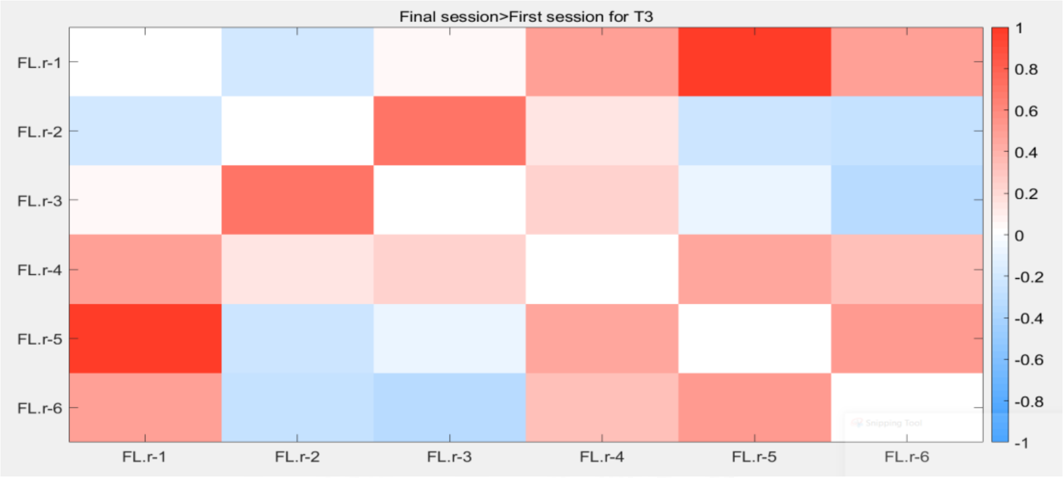


(b) Module Temporal Language

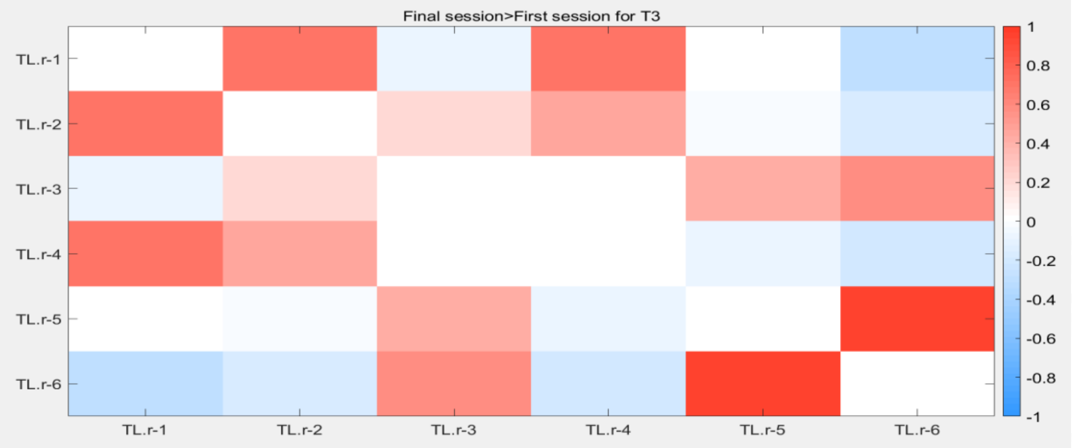


(c) Module Central Opercular

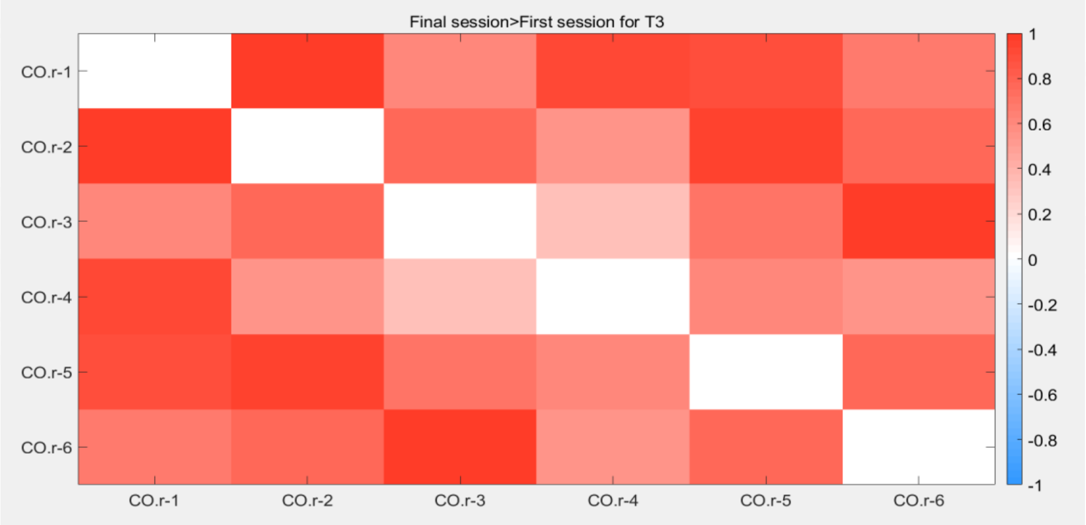
(d)Module Supra Parietal



(e) Module Frontal Language (right)



(f)Module Temporal Language (right)

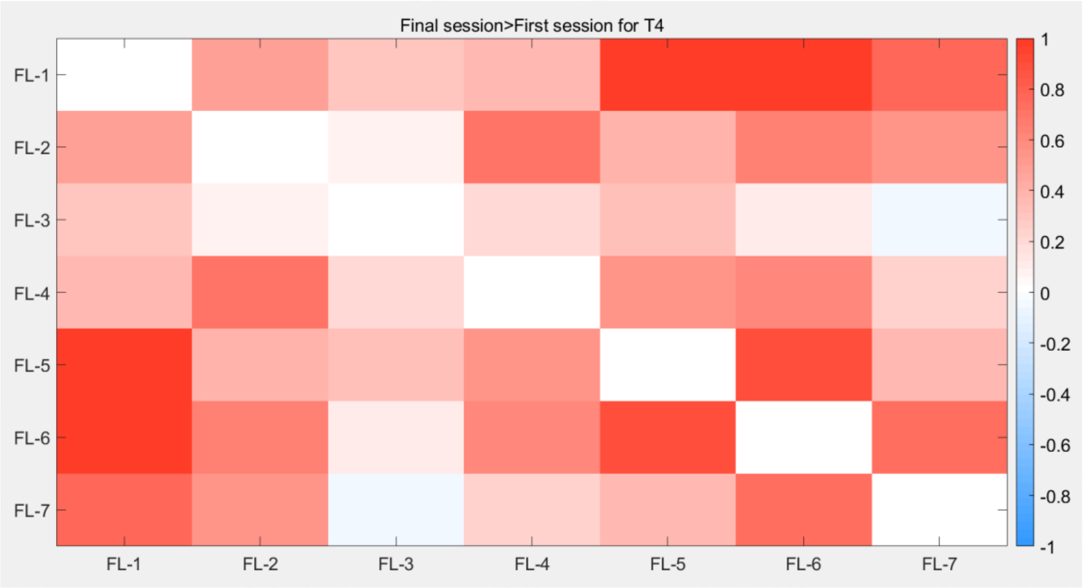


(g)Module Central Opercular (right)

Fig. 30: Intramodular Connectivity Matrix: Final Session > First Session for T3

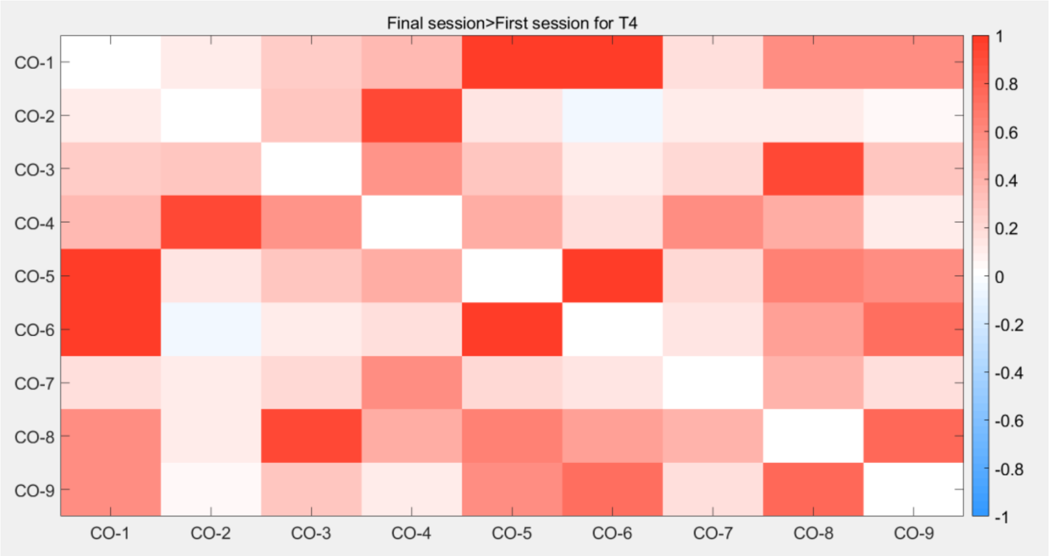
($p<0.05$(*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****); The colour bar indicates the strength between pairs of ROIs.)

For the third patient (T3), many ROIs in the frontal language, the temporal language, the central opercular, the supra parietal, the frontal language (right), the temporal language (right), and the central opercular (right) modules show good FC among themselves (Figure 30 (a), (b), (c), (d), (e), (f), (g)).

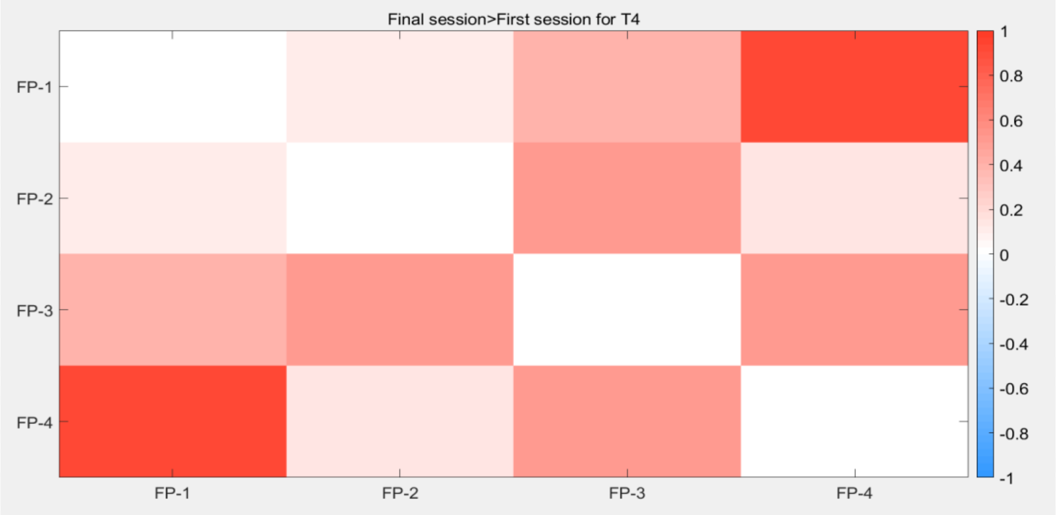


(a)Module Frontal Language

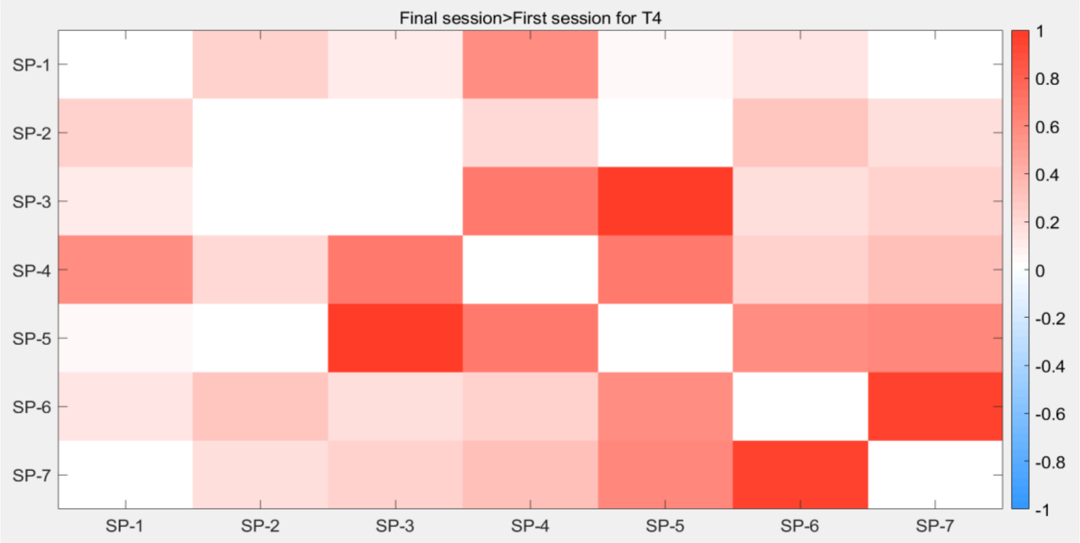
(b)Module Temporal Language



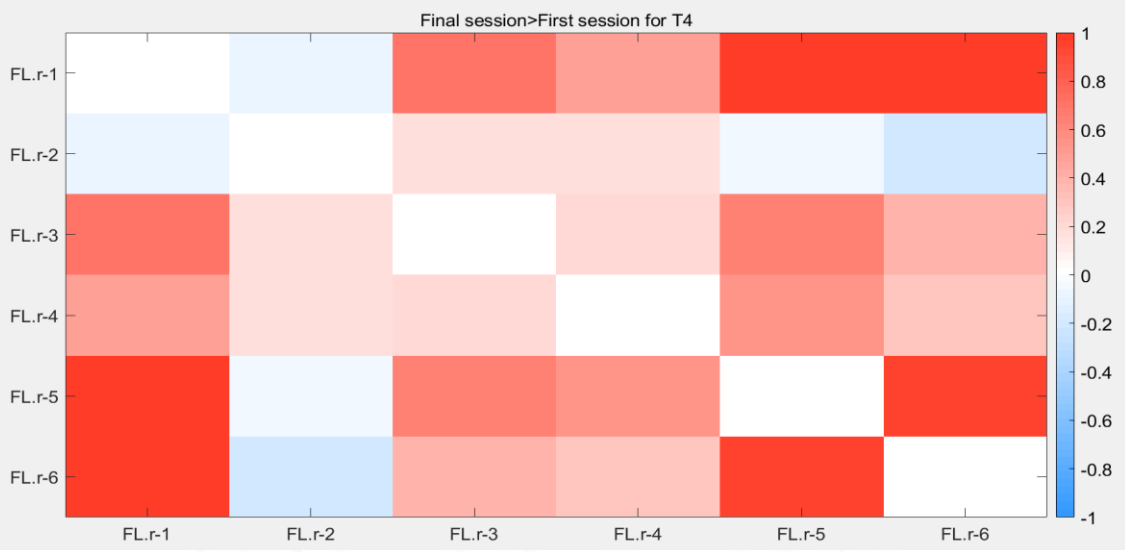
(c)Module Central Opercular



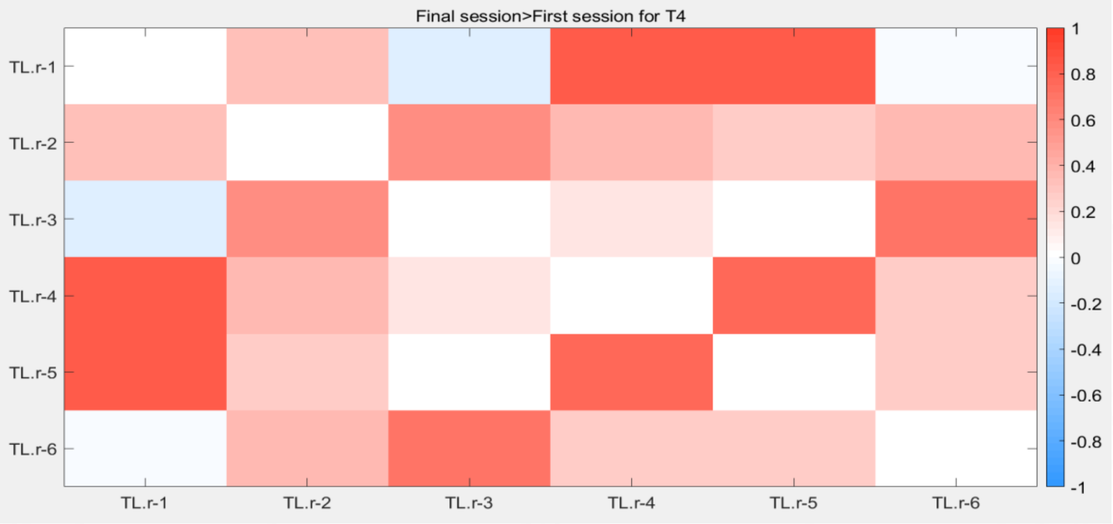
(d)Module Frontal Polar



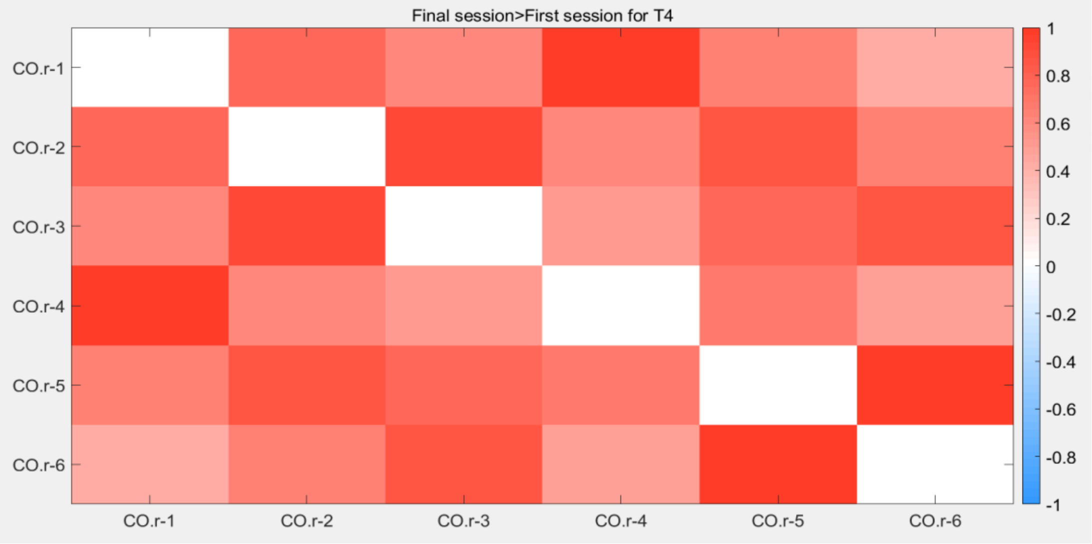
(e)Module Supra Parietal



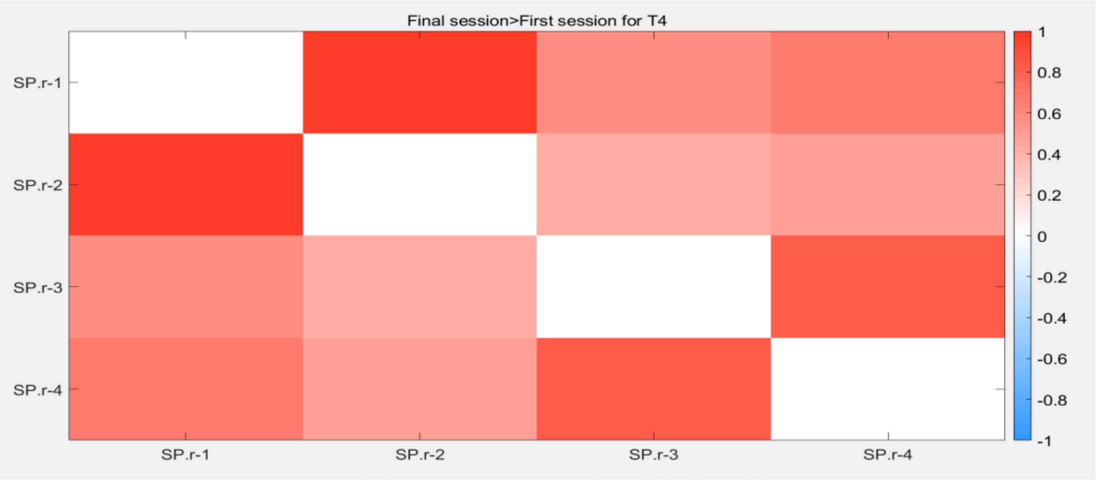
(f)Module Frontal Language (right)



(g)Module Temporal Language (right)



(h)Module Central Opercular (right)



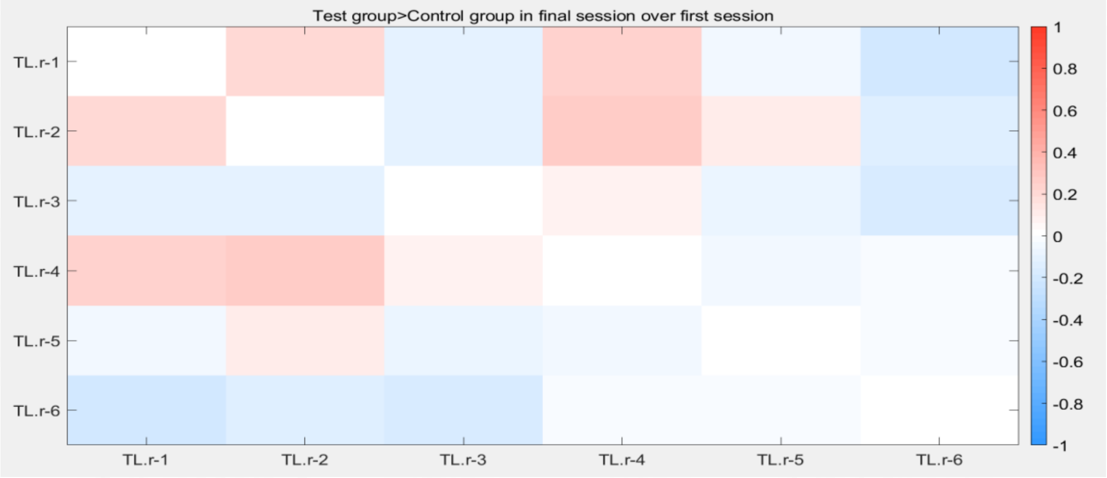
(i)Module Supra Parietal (right)

Fig. 31: Intramodular Connectivity Matrix: Final Session > First Session for T4

($p<0.05$(*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****); The colour bar indicates the strength between pairs of ROIs.)

In the fourth patient (T4), ROIs in the frontal language, the temporal language, the central opercular, the frontal polar, the supra parietal, the frontal language (right), the temporal language (right), the central opercular (right), and the supra parietal (right) modules have good FC (Figure 31 (a), (b), (c), (d), (e), (f), (g), (h), (i)).

Intramodular connectivity matrices with no significant result have not been included in this thesis. (Example, Figure 32 (a)).



(a) Module Temporal Language (right)

Fig. 32: Intramodular Connectivity Matrix: Test group > Control group in final session over first session

($p<0.05$(*), $p<0.01$(**), $p<0.001$(***), $p<0.0001$(****); The colour bar indicates the strength between pairs of ROIs. Connectivity matrices without any significant result have not been included in the thesis.)

CHAPTER 5

DISCUSSION

On analysing the results of the experiment, it was observed that functional connectivity in the language areas of the brain were up-regulated well by the normal subjects. The test and control groups were also able to increase the functional connectivities, however, to a much lesser extent, when compared to the normal group.

5.1. INTRAMODULAR FUNCTIONAL CONNECTIVITY

The following interpretations can be drawn by observing the results of the study that are obtained in the form of intramodular connectivity matrices between ROIs:

I. Final sessions of Normal, Test, and Control groups:

- During the 6th sessions of the normal, and the test groups, and during the 2nd session of the control group, the left hemispheric ROIs showed strong functional connectivity among themselves, when compared with the right hemispheric ROIs.
- ROIs present in the temporal language module, and the supra parietal module and it's right homologue, were observed to have good connectivity during the last sessions of the three groups, which suggests that these three modules were actively involved in language performance, in the three groups, by the end of the training.
- Specifically, ROIs posterior supramarginal gyrus (pSMG), and Angular gyrus (AG) of the temporal language module, and ROIs right homologue of precentral gyrus (PreCG.r), and right homologue of postcentral gyrus (PostCG.r) of the right hemispheric supra parietal (right) module, were observed to have strong FC with other ROIs in their respective modules during the final session of the training.

- The subjects in the normal and control groups showed good functional connectivity in a greater number of modules (in both left, and right hemispheres), as compared to those in the test group who showed good functional connectivity only in the left hemispheric modules.

II. Inter-group comparisons:

- In the comparison of the normal group over test group, under different conditions, only left hemispheric ROIs were found to have good functional connectivity. Specifically, ROI middle orbital gyrus (FrontalMidOrb_L) of the Frontal Polar module was observed to have good connectivity with other ROIs in that module, which suggests that this region which is actively involved in language performance in normal subjects was weak in test patients.
- In the comparison of the test group over the control group, ROIs in the left hemispheric supra parietal module, and the right hemispheric central opercular (right) module showed strong functional connectivity among themselves. Specifically, ROIs precentral gyrus (Precentral_L), right central operculum (CO.r), and parietal operculum (PO.r), showed enhanced connectivity with other ROIs in their respective modules, which suggests that with the neurofeedback training the test patients were able to up-regulate activities in these regions, more than the control patients who did not receive any training.

III. Intra-group comparison:

- In the intra-group comparison for test patients, it was observed that both left and right hemispheric ROIs displayed rise in functional connectivity. Among these, ROIs in the left hemispheric modules of supra parietal and central opercular showed enhanced connectivity in most of the comparisons. ROIs precentral gyrus (PreCG), and postcentral gyrus (PostCG) of the supra parietal module, and ROIs central operculum

(CO), and Heschl's gyrus (HG) of the central opercular module were observed to have the strongest connectivity with other ROIs in their respective modules.

- In the case of intra-group comparison for the normal subjects, it was observed that the left hemispheric ROIs were more active than the right hemispheric ROIs. ROIs in the central opercular module, and in the right hemispheric temporal language module, were specifically found to be strongly connected.

IV. Individual test patients:

- In three out of the four test patients, ROIs in the central opercular module are functionally well connected among themselves.

5.2. LIMITATIONS OF THE STUDY

1. In this study up-regulation task could be performed by subjects for small periods. Each session comprised of about 10 minutes of up-regulation. Moreover, the sessions could be performed only at an interval of one week. Less duration of up-regulation could be the reason behind the absence of significant improvement in the language performance of patients. Speech and language therapy (SLT) has been observed to be effective if approximately 7.5 hours of therapy is given per week for at least 2 weeks (Harnish et al., 2008).
2. A longer and more intense training may also show the development of functional connectivity changes more accurately and it's involvement in restoring language activity.
3. The small sample size of four subjects in each of the normal, test, and control groups, in the study, was due to the low number of patients that could be screened in and participate in the entire duration of the experiment.
4. The lesions did not match in the patients in their size and extent, though they were diagnosed with Broca's aphasia.
5. The language tests that are assigned should be such that they are more sensitive to the behavioural effects pertaining to language activity that are brought about by the training.

5.3. FUTURE SCOPE

1. Rehabilitative training can be tuned to activate the regions that have been observed to show a rise in functional connectivity in this study.
2. Less expensive modalities such as electroencephalograph and functional near-infrared spectroscopy can be assigned in further studies.

CONCLUSION

Neurorehabilitation by brain plasticity in aphasic stroke patients can be due to the recovery of language areas, or the restoration of the connections within these areas either as originally or via new connections bypassing the damaged ones. This study analysed the application of real-time fMRI-based neurofeedback training for the rehabilitation of post-stroke patients with expressive aphasia. Real-time fMRI generates data on brain activity, which in this study has been visually presented to the normal, the test, and the control subjects being scanned to form a neurofeedback loop in order to enhance the self-regulation of BOLD activity in specific brain regions.

The study shows that:

- with the neurofeedback training the test patients were able to increase functional connectivities among the ROIs in the left hemispheric supra parietal module, and the right hemispheric central opercular (right) module like, ROIs precentral gyrus (Precentral_L), right central operculum (CO.r), and parietal operculum (PO.r), more than the control patients who did not receive any training.
- ROIs in the left hemispheric modules of supra parietal and central opercular, like, ROIs precentral gyrus (PreCG), and postcentral gyrus (PostCG) of the supra parietal module, and ROIs central operculum (CO), and Heschl’s gyrus (HG) had the most significant rise in connectivity with other ROIs in their respective modules, in the test group.

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APPENDIX: Script for obtaining connectivity matrix

```
% Inputs - Second level Result Directory - ROI.mat(y, names, names2), INDSORT
% Outputs - Matrix of connectivity scores between modules during each
%         condition / comparison
%       - Matrix of T statistic for ROIs
%       - The Output varibales have to be copied to an excel sheet and
%         sorted as per module to get the BrainNet output

clear all; close all;

% The 2nd level results folder to be processed
%conn_dir='I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals\rest’;
%conn_dir='I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals(1).Tests(-1)\rest';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals(1).Tests(-1)\s1';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals\2ndhalfgt1sthalf';
% conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals\s1(-1).s6(1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals\UR(1).BL(-1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\s1(-1).s2(-1).s3(-1).s4(1).s5(1).s6(1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\UR(1).BL(-1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\s1(-1).s6(1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\S6.gt.S1.UR.gt.BL';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\S1_UR(-1).S6_UR(1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\S1_BL(-1).S6_BL(1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests(1).Controls(-1)\s1(-1).s6(1)';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\TgtCS6gtS1URgtBL';
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests(1).Controls(-1)\S1_BL(-1).S6_BL(1)';
%conn_dir = 'F:\SUJ-PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Tests\s6'; %s1 - s6
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Normals\s6'; %s1 - s6
%conn_dir = 'I:\SUJ\PhD\conn_NTC3\results\secondlevel\ANALYSIS_02\Controls\s6'; %s1,s6
```

```
%Is the comparison within group(0) or between groups(1)
COMP = 0;

%Number of subjects, 4 if only one group, 8 if two groups are studied
%together
if(COMP)
   NSUB = 8; %between two groups
else
   NSUB = 4; %within group
end

%Number of clusters, 6 on LH and 6 on RH
NCL = 12;

%Number of ROIs studied under the set Language ROIs
NROI = 65;

%load the connectivity matrices, source names, target names
clear conn_results;
clear conn_matrix;

conn_file = fullfile(conn_dir, 'ROI.mat');

load(conn_file, 'ROI'); %var ROI loaded

% Looking at first 65 ROIs alone - chosen as involved in language for study
%Read all the correlation scores into the a_corr matrix, the last index is
%the patient number
corr = zeros(NROI, NROI, NSUB);

intercon = zeros(NCL, NCL, NSUB);

if ndims(ROI(1,1).c2) == 2
   c2 = ROI(1,1).c2;
```

```
else
   c2 = 1;
end

%% Insert all connectivity scores for each patient into the corr matrix
for i = 1:65 %loop over source ROIs

   for j = 1:NSUB %loop over group members
      corr(i, :, j) = c2*squeeze(ROI(1,i).y(j,1:NROI,:))'; %y is the connectivity for all ROIs to the ith ROI
   end
end

for k = 1:NSUB
   corr(:,:,k)=weight_conversion(corr(:,:,k),'autofix');     % Set main diagonal to zero, remove any NaN
(or Inf values) , correct round-off errors
end

%roin = [1:NROI]; % only 65 ROIs chosen, removed SMA r&l

%% Load FC_data %%
% data=Z_mean(roin, roin);

%  roi_names=ROI.ROI(1,1).names(1:136);
roi_names=ROI(1,1).names(1:NROI);
roi_locs=ROI(1,1).xyz(1:NROI);

cl_sort = [1 2 7 8 1 6 3 3 4 4 6 2 6 2 3 10 4 9 3 7 1 7 1 7 1 7 1 12 6 11 ...
   5 11 5 8 2 11 5 8 2 8 2 12 6 12 6 12 6 8 2 8 2 10 4 7 1 9 3 9 3 9 3 9 ...
   3 9 3];

CL_IND = [1 5    21      23      25      27      55      2       12      14      35      39      41
         49      51      7       8 ...
   15  19        57      59      61      63      65      9       10      17      53      31      33
         37      6       11      13      29 ...
```

```
  43   45     47     3      20     22     24     26     54     4      34     38     40
       48     50     18     56     58 ...
  60   62     64     16     52     30     32     36     28     42     44     46]';

roi_names = {'FL-1';'TL-1';'FL.r-1';'TL.r-1';'FL-2';'SP-1';'CO-1';'CO-2';...
  'FP-1';'FP-2';'SP-2';'TL-2';'SP-3';'TL-3';'CO-3';'FP.r-1';'FP-3';'CO.r-1';'CO-4';...
  'FL.r-2';'FL-3';'FL.r-3';'FL-4';'FL.r-4';'FL-5';'FL.r-5';'FL-6';'SP.r-1';'SP-4';'TP.r-1';'TP-1';...
  'TP.r-2';'TP-2';'TL.r-2';'TL-4';'TP.r-3';'TP-3';'TL.r-3';'TL-5';'TL.r-4';'TL-6';...
  'SP.r-2';'SP-5';'SP.r-3';'SP-6';'SP.r-4';'SP-7';'TL.r-5';'TL-7';'TL.r-6';'TL-8';'FP.r-2';'FP-4';'FL.r-6';'FL-7';'CO.r-2';'CO-5';'CO.r-3';'CO-6';'CO.r-4';'CO-7';'CO.r-5';'CO-8';'CO.r-6';'CO-9'};

cl_names = {'FL'; 'TL'; 'CO'; 'FP'; 'TP'; 'SP'; 'FL.r'; 'TL.r'; 'CO.r'; 'FP.r'; 'TP.r'; 'SP.r'};

MOD1 = [1   5    21     23     25     27     55]; %FL
MOD2 = [2        12     14     35     39     41     49     51]; %TL
MOD3 = [7   8   15  19  57     59     61     63     65]; %CO
MOD4 = [9        10     17     53]; %FP
MOD5 = [31       33     37]; %TP
MOD6 = [6        11     13     29  43   45     47];%SP
MOD7 = [3        20     22     24     26     54]; % FL.r
MOD8 = [4        34     38     40     48     50]; % TL.r
MOD9 = [18       56     58  60   62     64]; % CO.r
MOD10 = [16      52]; % FP.r
MOD11 = [30      32     36]; % TP.r
MOD12 = [28      42     44     46]; %SP.r

MOD = {MOD1, MOD2,MOD3, MOD4, MOD5, MOD6, MOD7, MOD8, MOD9, MOD10, MOD11, MOD12};

%% Connectivity Measure - For each subject in a particular condition

intercon = zeros(NCL, NCL, NSUB);
  for k = 1:NSUB
      mask = zeros(size(corr(:,:,k)));
```

```
      for i = 1:NCL
         cli = cl_sort==i;

         if sum(cli) > 0
            for j = 1:NCL
               clj = cl_sort==j;

               if sum(clj) > 0
                  mask = double(cli)'*double(clj);

                  %mask has 1 where a required connection exists between Mod i
                  %and Mod j
                  mod_con = corr(:,:,k).*mask;
                  intercon(i,j,k) = sum(sum(mod_con));
               end
            end
         end
      end
   end




t_con = zeros(NROI);
p_con = zeros(NROI);
m_con = zeros(NROI);

for i = 1:NROI
  for j = 1:NROI
      if(COMP)
         [h, p] = ttest2(corr(i,j,1:NSUB/2), corr(i,j,NSUB/2+1:NSUB));
      else
         [h,p] = ttest(corr(i,j,:));
```

```
        end
         t_con(i,j) = h;
         p_con(i,j) = p;
         if(COMP)
            m_con(i,j) = mean(corr(i,j,1:NSUB/2))-mean(corr(i,j,NSUB/2+1:NSUB));
         else
            m_con(i,j) = mean(corr(i,j,1:NSUB));
         end
         %f=f+1;
      end
   end
%   %INDIVIDUAL SUBJECTS
 %MOD1_to_MOD1_connectivity=zeros(length(MOD12),length(MOD12));

 %row=0;
 %column=0;

 %for i=MOD12
  %  row=row+1;
    %for j=MOD12
       % column=column+1;

        %MOD1_to_MOD1_connectivity(row,column)=corr(i,j,4);



    % end
    % column=0;
    % end
% figure;
 % imagesc(MOD1_to_MOD1_connectivity);

 % hold on;  % hold on to overlay community visualization

%load('MyColormaps','mycmap');
```

```
%set(gcf,'Colormap',mycmap)
%colorbar;

%caxis([-1 1])
%lim=caxis;

%  xlabel=[];
%ylabel=[];
%for i=MOD12
  %  xlabel=[xlabel roi_names(i)];
%end

%for i=MOD12
%    ylabel=[ylabel roi_names(i)];
%end
%set(gca,'YTickLabel', ylabel);



%set(gca, 'YTick', [1:length(ylabel)]);

%set(gca,'XTickLabel', xlabel);
%set(gca, 'XTick', [1:length(xlabel)]);
%set(gca,'FontSize', 16);

%title('Final session>First session for T4');

%   %GROUPWISE
            MOD1_to_MOD1_connectivity=zeros(length(MOD6),length(MOD6));
        c=0;
        row=0;
        column=0;

        for i=MOD6
```

```
     row=row+1;
     for j=MOD6
        column=column+1;

        MOD1_to_MOD1_connectivity(row,column)=m_con(i,j);
        c=c+1;

     end
     column=0;
     end
 figure;
  imagesc(MOD1_to_MOD1_connectivity);

  hold on;  % hold on to overlay community visualization

load('MyColormaps','mycmap');
set(gcf,'Colormap',mycmap)
colorbar;

caxis([-1 1])
lim=caxis;

  xlabel=[];
ylabel=[];
for i=MOD6
   xlabel=[xlabel roi_names(i)];
end

for i=MOD6
   ylabel=[ylabel roi_names(i)];
end
set(gca,'YTickLabel', ylabel);
```

```
set(gca, 'YTick', [1:length(ylabel)]);

set(gca,'XTickLabel', xlabel);
set(gca, 'XTick', [1:length(xlabel)]);
set(gca,'FontSize', 16);

title('Final session of Test group');
%pv = p_val(x,y);
%To create the p-value for each connection (i,j)
row=0;
column=0;
for i =MOD6
   column=0;
   row=row+1;
   for j = MOD6
      column=column+1;
      pv_array(row,column) = p_con(i,j);
   end
end
for i=1:length(MOD6)
   for j=1:length(MOD6)
      pv=pv_array(i,j);
      if pv < 0.0001
         pstr = '****';
      else if pv < 0.001
            pstr = '***';
          else if pv < 0.01
                pstr = '**';
             else if pv <0.05
                   pstr = '*';
                else
                   pstr = '';
                end
```

```
            end
         end
       end
       text(j,i,pstr,'FontSize',16,'HorizontalAlignment','center');


    end
end
```