

CHANGES OF BRAIN NETWORK CONNECTIVITY AMONG DIFFERENT
HUMAN VIGILANCE STATES INVESTIGATED BY EEG

by

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ABSTRACT

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Understanding the brain network connectivity associated with human vigilance states, to investigate the basic neural processes that underlie complex higher-order cognitive operations and functional domains, has become an important topic in biological science research. Also, there is growing concern about the prevalence and effects of sleep deprivation. Given the increasingly fast-paced nature of our society, the focus of the researchers is not only to identify the changes occurring within the brain during sleeping stages, but also to extract meaningful psycho-behavioral reason behind such change patterns which can lead to an improved understanding of brain activity during sleep which may further help in diagnosing various sleep disorders resulting in abnormal connectivity.

In this study, I performed data analysis of 64-channel electroencephalogram (EEG) taken from 18 human subjects to explore changes of brain network connectivity during different vigilance states, meaning when a person gradually shifts from eyes open to just eyes closed and later to sleep stages 1 and 2. A correlation method, a linear connectivity measure implemented using a free-downloadable software package, Brainstorm, was used to investigate the human brain functional connectivity in four frequency bands (i.e., delta, theta, alpha and beta band), under four vigilance states: eyes open, eyes closed, sleep stage 1, and sleep stage 2. Comparative analyses of the network properties and patterns of connection between the 4 stages were done using EEG topo plot analysis, a method for evaluating the similarity between signals in the frequency domain.

The pooled connectivity plots across all four bands show the location and number of significant connectivity changes in between different vigilance stages. Also, electrode-based topography across the entire human heads provides in-depth results, showing prevalent network connections and changes in long or short distances during transition from one vigilance state to another. My study reported meaningful convergence with previous sleep studies; my observations in each transition phase were discussed with speculations based on pre-existing researches.

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CHAPTER 1

INTRODUCTION

1.1 What Is an EEG?

An **electroencephalogram (EEG)** is a non-invasive method to measure the electrical activity of a large number of synchronously firing neurons in our brain, to investigate the basic neural processes that underlies the complex higher-order cognitive operations and functional domains. EEG measures neurophysiological function during different tasks and conditions. The applications of this method are extremely wide-spread, as there are nearly infinite number of domains of interest which can be explored to better understand the relative timing of neural events.

EEG tracks and records brain wave patterns. Small electrodes with flat metal discs that are attached to the scalp with wires analyze the brain electrical impulses. These signals are recorded and displayed by a computer. The electrical impulses in an EEG recording look like wavy lines with peaks and valleys which allow doctors to quickly assess whether there are any irregularities such as seizures or other brain disorders consisting of abnormal EEG patterns.

1.2. What is the Human Brain?

- “The brain is an amazing three-pound organ that controls all functions of the body, interprets information from the outside world, and embodies the essence of the mind and soul. Intelligence, creativity, emotion, and memory are a few of the many things governed by the brain. Protected within the skull, the brain is composed of the cerebrum, cerebellum, and brainstem. The brainstem acts as a relay center connecting the cerebrum and cerebellum to the spinal cord”. [1]
- The **cerebrum** which comprises the largest part of the brain and is composed of right and left hemispheres. It performs higher functions like interpreting touch, vision and hearing, as well as speech, reasoning, emotions, learning, and fine control of movement.

- The **cerebellum**, the coordinator of the muscle movements, maintain posture, and balance, is located under the cerebrum.
- “The **brainstem** includes the midbrain, pons, and medulla. It acts as a relay center connecting the cerebrum and cerebellum to the spinal cord. It performs many automatic functions such as breathing, heart rate, body temperature, wake and sleep cycles, digestion, sneezing, coughing, vomiting, and swallowing. Ten of the twelve cranial nerves originate in the brainstem”. [1]

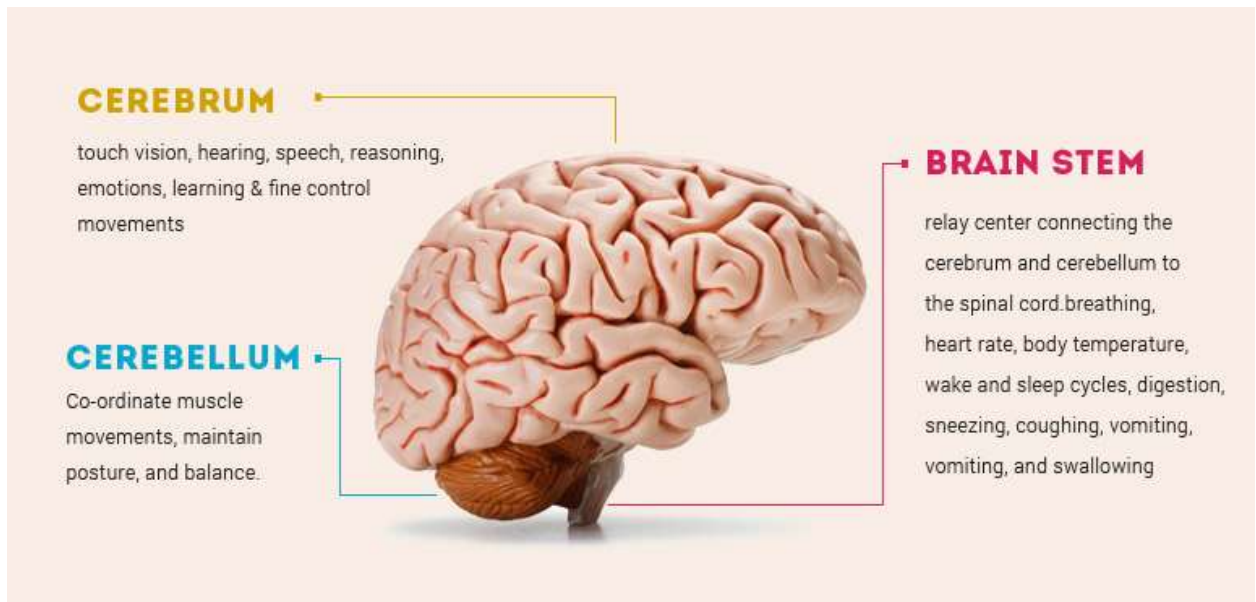


Fig 1.1: Different parts of brain and their functions [Reference: <http://humanbrainfacts.org/human-brain-anatomy.php>]

Cortex is the folded type appearance on the surface of the cerebrum which about 70% of the 100 billion nerve cells. The color the Cortex is colored grey-brown by these nerve cell bodies giving the name gray matter. Axons which act as long connecting fibers between neurons and located beneath the cortex make up the white matter.

1.2.1. Right brain – left brain

The right and left hemispheres of the brain are joined by a bundle of fibers called the corpus callosum that delivers messages from one side to the other. Each hemisphere controls the opposite side of the body. So, a brain tumor located on the right side of the brain can potentially weak or paralyze the left arm or leg.

Not all functions of the hemispheres are shared. In general, the right hemisphere controls creativity, spatial ability, artistic, and musical skills whereas the left hemisphere which is responsible for speech, comprehension, arithmetic, and writing is dominant in hand use and language in about 92% of people. [1]

1.2.2. Lobes of the brain

“The cerebral hemispheres have distinct fissures, which divide the brain into lobes. Each hemisphere has 4 lobes: frontal, temporal, parietal, and occipital. Each lobe may be divided, once again, into areas that serve very specific functions. It’s important to understand that each lobe of the brain does not function alone. There are very complex relationships between the lobes of the brain and between the right and left hemispheres”. [1]

1.2.2.1. Frontal lobe

- Personality, behavior, emotions
- Judgment, planning, problem solving
- Speech: speaking and writing (Broca’s area)
- Body movement (motor strip)
- Intelligence, concentration, self-awareness

1.2.2.2. Parietal lobe

- Interprets language, words
- Sense of touch, pain, temperature (sensory strip)
- Interprets signals from vision, hearing, motor, sensory and memory
- Spatial and visual perception

1.2.2.3. Occipital lobe

- Interprets vision (color, light, movement)

1.2.2.4. Temporal lobe

- Understanding language (Wernicke's area)
- Memory
- Hearing
- Sequencing and organization

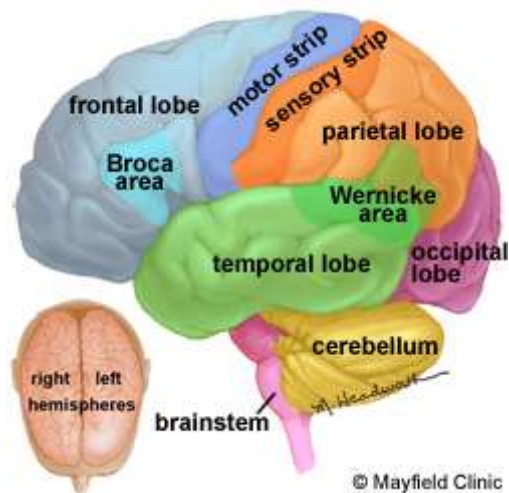


Fig 1.2: Different regions in brain [Reference: <https://www.mayfieldclinic.com/PE-AnatBrain.htm>]

1.3. What is sleep?

“Sleep is not an easy concept to define. It is not simply the passive absence of wakefulness. We all know what it is from personal experience, yet it would be very difficult to explain the nature of sleep to, say, some recent visitor from another planet with utterly no experience with this nightly habit of ours.

Sleep is generally thought of as a suspension of normal consciousness, especially since normal sleepers are very poor witnesses to their own sleep experiences, and many environmental stimuli simply fail to register with a sleeper. As usual, there are always exceptions. For example, take the old idea that a sleeping mother with a newborn infant can sleep through many strange noises, but awakens quickly if her baby cries in the night. Scientific studies have shown that sleeping persons can process information during sleep, ignoring some sounds and responding to others while remaining asleep". [2][3]

1.3.1. Why is sleep study important?

"We know that sleep significantly affects the brain and the rest of the body, and that it is important for physical and emotional health and well-being. Yet, we know surprisingly little about sleep's specific purpose and function — other than observing that people are less tired, and function better, after a good night's sleep, and feel worse and function poorly when they don't sleep well. Sleep is important in the consolidating learning and memories, and may be important in maintaining brain health as we age. Only in the last 30 years have physicians and scientists systematically explored sleep disorders.

Unfortunately, individuals who do not get adequate sleep lose insight into the effects the sleep deprivation is having on their day-to-day functioning. Only when their sleep problems are corrected do they realize how bad they had it. Given the increasingly fast-paced nature of our society, there is growing concern about the prevalence and effects of sleep deprivation". [2][3]

1.3.2. Stages of Human Sleep

Following the discovery of rapid eye movement (REM) sleep in 1953, researchers learned that there are three basic states of consciousness: wakefulness, REM sleep, and non-rapid eye movement (NREM) sleep.

1.3.2.1. REM sleep is "an active period of sleep marked by intense brain activity. Brain waves are fast and desynchronized, similar to those in the waking state. Breathing becomes more rapid, irregular, and shallow; eyes move rapidly in various directions and limb muscles become temporarily paralyzed. Heart rate

increases and blood pressure rises. This also is the sleep stage in which most dreams occur.” REM sleep is thought to play a role in memory consolidation, the synthesis and organization of cognition, and mood regulation.

1.3.2.2. NREM sleep is characterized by a reduction in physiological activity. As sleep deepens, a person’s brain waves slow down and gain amplitude, both breathing and the heart rate slow down, and the individual’s blood pressure drops. NREM sleep consists of three stages:

- *N1* (formerly “stage 1”) is a time of drowsiness or transition from being awoken to falling asleep. Brain waves and muscle activity begin slowing down in this stage. People in *N1* sleep may experience sudden muscle jerks, preceded by a falling sensation.
- *N2* (formerly “stage 2”) is a period of light sleep during which eye movements stop. Brain waves become slower, with occasional bursts of rapid waves (called sleep spindles) and spontaneous periods of muscle tone mixed with periods of muscle relaxation. The heart rate slows and body temperature decreases.
- *N3* (formerly “stages 3 and 4”) is called “slow wave sleep” (SWS) and is characterized by the presence of slow brain waves called “delta waves” interspersed with smaller, faster waves. Blood pressure falls, breathing slows, and temperatures drops even lower, with the body becoming immobile. Sleep is deeper, with no eye movement and decreased muscle activity, although muscles retain their ability to function. [2][3]

1.4. Abnormal Sleep & Sleep Disorders:

“Sleep disorders are among the most common medical complaints in our society. The National Sleep Foundation’s (NSF) 2005 *Sleep in America Poll* indicated that 75 percent of adults surveyed reported having at least one symptom of a sleep problem at least a few nights a week — an increase from 62 percent in 1999, 69 percent in 2000, and 74 percent in 2002. [4][5] Millions of Americans suffer from sleep problems, including:

- **1.4.1.** Insomnia is the most common sleep complaint, involves trouble getting to sleep and/or staying asleep, and/or experiencing unrefreshing sleep. About 30-40 percent of adults report some insomnia symptoms in any given year; about 10-15 percent of adults say they have chronic insomnia. In fact, most insomniacs often have some sort of medical or psychic problem that is co-morbid with insomnia, such as anxiety, depression, substance abuse, diabetes, Alzheimer's disease, and/or chronic pain. [4][5]
- **1.4.2.** Obstructive sleep apnea (OSA) is the most common form of sleep apnea. It occurs when the person's airway collapses or is blocked (either totally or partially), which causes shallow breathing or a complete stoppage in breathing, thereby disrupting sleep. It is estimated or thought that 4% of U.S. men, and 2% of U.S. women have OSA. Studies in other countries report similar prevalence rates. Most OSA is undiagnosed, however. [4][5]
- **1.4.3.** Circadian Rhythm Disorders involves problems with an individual's internal clock that disrupt his or her sleep patterns. *Shift work disorder* (SWD) is a form of Circadian Rhythm Disorder with serious medical and psychiatric consequences; it is predominantly experienced by those who work night shifts and early morning shifts. Shift workers are more likely to suffer from insomnia and excessive daytime sleepiness compared to those who work during the day (61% vs. 47%, and 30% vs. 18% respectively). Shift workers are also more likely to drive while fatigued and to fall asleep at the wheel. [4][5]
- **1.4.4.** Parasomnia refers to all of the abnormal movements or activities that can happen while people sleep (other than sleep apnea), including sleep-related abnormal movements, behaviors, emotions, perceptions, and dreams that occur while falling asleep, sleeping, between sleep stages, or during arousal from sleep. They include eating disorders, sleepwalking, sleep talking, nightmares, sleep paralysis, REM sleep behavior disorder, and sleep aggression. Parasomnias affect about 10 percent of Americans and are more common in children due to their brain immaturity. [4][5]

- **1.4.5.** Restless legs syndrome (RLS) is a neurological disorder that causes uncomfortable (and sometimes painful) tingling and tugging sensations in the legs. RLS symptoms usually come on in the evening and intensify as the night goes on. Movement of the limbs instantly relieves the discomfort, then being still once again makes them worse. RLS almost always interferes with one's ability to fall asleep and to stay asleep. Most people with this disorder feel tired during the day, since RLS interferes with sleep's restorative process. RLS is often undiagnosed or misdiagnosed; about 10 percent of American adults suffer from RLS, fewer among younger people, more among the elderly. [4][5]

- **1.4.6.** Excessive sleepiness disorder is persistent sleepiness (what is often referred to as "tiredness"), which interferes with a person's productivity and quality of life. It can result from (1) insufficient sleep (e.g., insomnia), (2) poor quality sleep (e.g., sleep apnea), (3) erratic sleep patterns (e.g., shift work disorder), (4) medications and substances (e.g., prescription and over-the-counter medications, drugs, and "natural" remedies), and (5) brain damage (e.g., traumatic brain injury, narcolepsy). According to NSF's 2008 *Sleep in America* poll, in the last month, due to excessive sleepiness:
 - ✓ 36 percent of Americans have driven while drowsy or fallen asleep while driving; 29 percent have fallen asleep or become very sleepy at work;
 - ✓ 20 percent have lost interest in sex due to sleepiness; and
 - ✓ 14 percent have missed family events, work functions, and/or leisure activities in the past month due to excessive sleepiness." [4][5]

1.5. History of sleep research

"Ideally, development in any area of science should fall into two phases: basic and applied. And so, it was for sleep research, representing a model of how progress might best occur in any area. The two main phases should include (1) the first phase of basic research to study the fundamental nature of normal sleep and (2)

the second phase of application research to deal with practical problems of sleep disorders.

Sigmund Freud: A familiar name who needs little introduction here, except to remember that it was Freud who rekindled much of our interest in dreams and dreaming, a major topic of this course. Working over one hundred years ago, Freud's contributions were widely praised and criticized alike. Freud worked before the discovery of sleep brain waves or REM sleep, hence much of his writing about dreams is now out of date.

Hans Berger: A German physician who was the first to observe and report human brain waves in 1929, including alpha waves and the fact that brain waves changed with sleep onset. Thereafter in the mid-1930s several American researchers reported other brain wave components in the normal sleep cycle, owing to the influence of Berger.

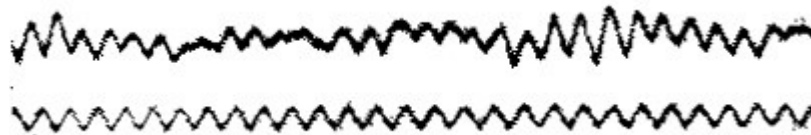


Fig 1.3: First EEG recorded by Hans Berger in 1929 [Reference: <http://online.missouri.edu/exec/data/courses/2360/public/lesson01/lesson01.aspx>]

Nathaniel Kleitman: A physiologist at the University of Chicago who was widely regarded as the world's leading sleep researcher for decades, both before and after World War II. It was in his laboratory that REM sleep was discovered. He authored a major sleep text that served as the primary reference in the area for decades.

Eugene Aserinsky: A graduate student in Kleitman's laboratory who, as a part of his master's thesis project, was the first to observe periods of rapid eye movements in sleeping infants in the early 1950s.

William Dement: Probably the world's second leading sleep researcher for decades. Following Aserinsky's discovery, Dement, with Kleitman, was the first to observe the high frequency of dream reports out of REM sleep. He founded the

first sleep clinic, and incidentally the area of sleep medicine, at Stanford University in 1970. He was also one of the first to recognize the significance of sleep apnea, and a number of other sleep disorders issues, which was the beginning of the applied phase in sleep work, known as sleep medicine. [6]

After that over the decades research works have been conducted to detect sleep disorders based on some salient features of EEG signal. Several EEG records have been collected for these sleep disorders and analyzed using discrete wavelet transform, Fast Fourier transforms etc.” [7]

Many different methods for automatic sleep stage classification have been proposed. These methods generally extract certain features of the signals to analyze the recording epoch and use a classification algorithm to identify the sleep stage. Several EEG-based automatic sleep stage detection methods using various feature extraction techniques have been published. These include time-domain analysis, frequency-domain analysis and time-frequency-domain analysis “In addition, nonlinear parameters and complexity measures have been used successfully. A wide range of machine learning-based methods such as Linear Discriminant Analysis (LDA), Artificial Neural Networks (ANN) Support Vector Machine (SVM), K-Nearest Neighbor (KNN) and Decision Trees (DT) have been proposed for classification problems, which have also been widely used for sleep stage classification.” [8]

“After that several research studies using the functional brain connectivity measures derived from electro-encephalogram (EEG) have been conducted to investigate the presence of autism of children during face perception tasks, classification of different emotional states and to investigate whether the neuronal network undergoes dynamic changes before and during the transition to an EEG epileptic discharge. Functional connectivity estimates the temporal synchrony among functionally homogeneous brain regions based on the assessment of the dynamics of topologically localized neurophysiological responses.” [9]

CHAPTER 2

SLEEP STUDY

2.1. Aim of my study and motivation behind it

Descent into sleep is accompanied by disengagement of the conscious brain from the external world. It follows that this process should be associated with reduced neural activity in regions of the brain known to mediate interaction with the environment. There is a physiologically distinct change in the state of the brain during sleep in comparison to wakefulness that is manifest subjectively as altered awareness and objectively as reduced responsiveness to environmental stimuli. Brain network connectivity was examined to evaluate defined networks for shifts in their interregional connectivity in 4 different brain states.

The human brain works through global communications across locally clustered regions that own their unit or unique functions. It means that the brain is functioned by global and local interactions between brain regions. According to various brain states, functional brain networks are dynamically reorganized and their patterns of connection are also changed. These functional brain reorganizations could be described by concept of functional segregation and integration. Considering sleep-awake regulations, recent studies have reported that the brain is regulated by changes of interaction between particular brain regions. Especially sleep stages are widely classified into NREM and REM sleep as detailed and identified into 4 stages. Thus, sleep is an appropriate topic to investigate changes of functional brain connectivity. In this study, I performed

EEG data analysis to explore changes of brain network connectivity during four different vigilance states, meaning when a subject gradually shifted from eyes open to just eyes closed and then later to sleep stages 1 and 2. Then I compared the network properties and patterns of connection between sleep and wakefulness using EEG topo plot analysis.

2.2. Materials and Methods

2.2.1. Ethics Statement

All subjects signed informed consent before the EEG experiments; the experimental protocol was approved by the Institutional Review Board (IRB) of the University of Texas Arlington.

2.2.2. Participants and Experimental paradigms

Participants were 18 healthy students from the University of Texas Arlington (10 males; 8 females) with their age ranging between 23-26 years. Individuals with a prior history of neurological or psychiatric illness or current or prior psychoactive medication use were excluded.

All participants participated voluntarily for a comprehensive dual-mode experiment consisting of 4 paradigms: 5-min finger tapping, 5-min eyes-open resting state, 10-min eye-closed/sleep state, and 5-min breath hold. The dual-mode brain scan system consisted of a 64-channel EEG unit and 133-channel functional near infrared spectroscopy device.

2.2.3. EEG Measurement

Encephalographic measurements employed an EEG recording system consisting of

- Bio semi 64 channel electrode cap with 64 AgCl electrodes
- amplifiers with filters
- A/D converter
- Recording device.

As shown in Fig. 2.1, the electrodes read the signals from the head surface, the amplifiers amplified the microvolt signals into the range where they could be digitalized accurately, the converter changed the signals from analog to digital forms, and the personal computer stored and displayed the obtained data.

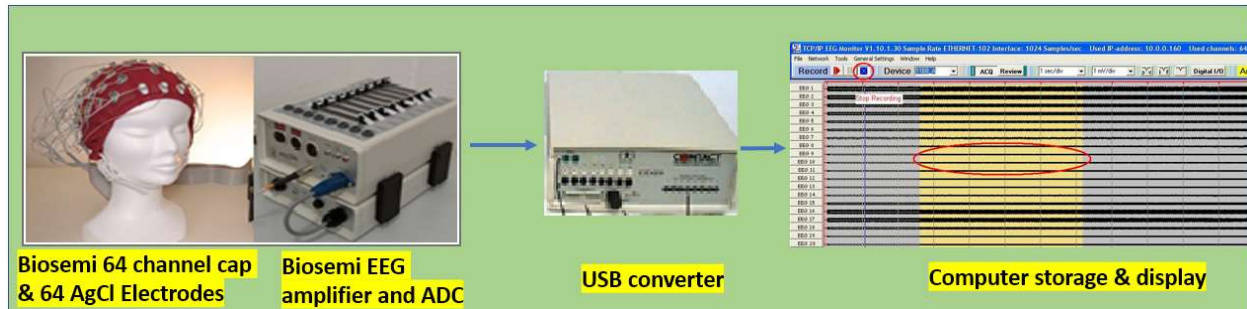


Fig 2.1: Experimental setup

In principle, “scalp recordings of neuronal activity in the brain, identified by the EEG, allow measurement of potential changes over time in basic electric circuit conducting between signal (active) electrode and reference electrode. The EEG recording electrodes and their proper functions are critical for acquiring appropriately high-quality data for interpretation. For multichannel montages, electrode caps are preferred, with number of electrodes installed on its surface.” [10]. In my study, EEG signals were recorded with 64-channel Biosemi equipment according to the international 10–10 system with 64 AgCl electrodes mounted on top of the subject’s head. Scalp electrodes consist of Ag-AgCl disks, 1 to 3 mm in diameter, with long flexible leads that can be plugged into an amplifier. AgCl electrodes can accurately record very slow changes in potential. The multi-channel configurations can comprise up to 128 or 256 active electrodes.

Using the silver-silver chloride electrodes, the space between the electrode and skin should be filled with conductive paste that also helps the electrode to stick. With a cap system, there is a small hole to inject the conductive paste, which serves as a medium to ensure small contact impedance at electrode-skin interface.

A ground electrode was attached to the center of the forehead which is needed as a reference point for getting differential voltages at all other electrode locations. Electrooculography (EOG) was measured to control for ocular artifacts. Vertical eye movement was measured using electrodes placed above and below

the left eye, and horizontal eye movement was measured with electrodes placed lateral to the left and right external canthi. EEG and EOG signals were amplified using a multichannel bio signal amplifier (band pass 0.1–100 Hz) and A/D converted at 500 Hz per channel with 12-bit resolution. The impedance of each electrode had to be less than 5 k Ω .

2.2.4. Experimental Procedure:

For the resting state to sleep state paradigm, the experiment began with a 30-s task where participants were shown a set of power-point-based slides on the monitor, asking them to be in a resting state with eyes open and make no other body part movements followed by a brief countdown to begin recording (from 5 to 1 with a step of 1 count per second), presented on the monitor. The eyes open resting state condition was recorded for 5 minutes long. At the end of 5 minutes, a second 30-s slide was shown to the participants on the monitor; this time it asked them to close their eyes given the freedom of falling asleep if they can. The monitor provided a second brief countdown to get them ready for eyes closed resting state recording (from 5 to 1 with a step of 1 count per second), presented on the monitor. The eyes closed resting state condition was recorded for 10 minutes long.

2.2.5. Signal Preprocess and Data Analysis

The EEG data were stored in the form of .bdf in the computer and then further processed using Brainstorm toolbox in MATLAB 2015b. “Brainstorm is a MATLAB-based, collaborative, and open-source application software package, dedicated to the analysis of brain recordings: MEG, EEG, fNIRS, depth electrodes and animal electrophysiology. This software was generated primarily with support from the National Institutes of Health. Primary support was also provided by the Centre National de la Recherche Scientifique (CNRS, France) for the Cognitive Neuroscience & Brain Imaging Laboratory (La Salpêtrière Hospital and Pierre & Marie Curie University, Paris, France), and by the Montreal Neurological Institute to the MEG Program at McGill University. Additional support was also from the French National Research Agency (ANR) and by the Epilepsy Center in the Cleveland Clinic Neurological Institute.” [11]

The main advantages of Brainstorm are:

- Rich and intuitive graphic interface.
- No programming knowledge needed.
- Data processing, production of cortical connectivity, and spatial-temporal EEG source estimation are all within the same program.

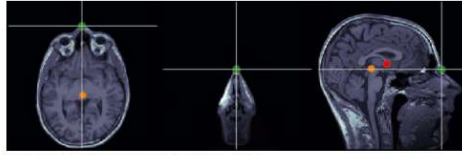
2.2.5.1. Importing the Subject Anatomy:

“For estimating the brain sources of the MEG/EEG signals with the correct anatomy of the subject, we must need at least three files: an MRI volume, the envelope of the cortex and the head surface of the head. The anatomical information given in Brainstorm was acquired with a 1.5T MRI scanner; the subject had a marker placed on the left cheek. The MRI volume was processed with Free Surfer 5.3. Number of vertices of the cortex surface: 15000 (default value).

This option defines the number of points that will be used to represent the cortex envelope. It was also the number of electric dipoles that I used to model the activity of the brain. This default value of 15000 was chosen empirically as a good balance between the spatial accuracy of the models and the computation speed.

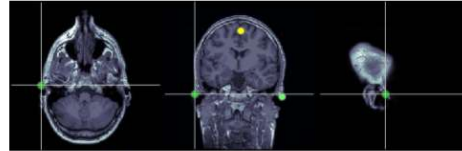
The MRI viewer was displayed followed by fiducial points selection where I needed to define the Subject Coordinate System (SCS): Nasion (NAS), Left ear (LPA), Right ear (RPA). See the following Fig. 2.2. This step was used to register the MEG/EEG sensors on the MRI.” [11] In a similar fashion, we define Anterior commissure (AC), Posterior commissure (PC) and any interhemispheric point (IH). See Fig. 2.3 below. This was used to align the individual subject's anatomy on the anatomical templates.

Nasion (NAS)



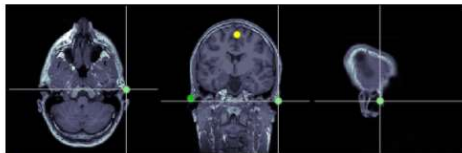
MRI coordinates: 127, 213, 139

Left ear (LPA)



MRI coordinates: 52, 113, 96

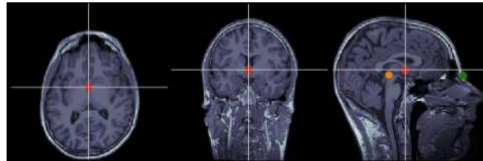
Right ear (RPA)



MRI coordinates: 202, 113, 91

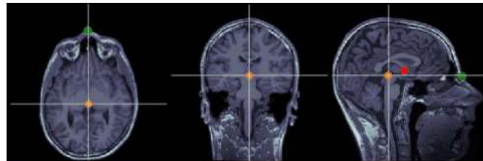
Fig 2.2: MRI co-ordinates of the NAS, LPA AND RPA used in subject anatomy [Reference: <http://neuroimage.usc.edu/brainstorm/Tutorials/ImportAnatomy>]

Anterior commissure (AC)



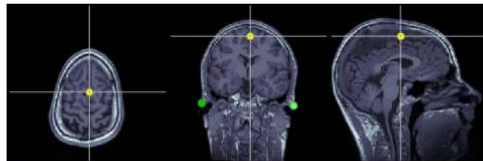
MRI coordinates: 127, 119, 149

Posterior commissure (PC)



MRI coordinates: 128, 93, 141

Inter-hemispheric point (IH)



MRI coordinates: 131, 114, 206

Fig 2.3: MRI co-ordinates of the AC, PC AND IH used in subject anatomy. [Reference: <http://neuroimage.usc.edu/brainstorm/Tutorials/ImportAnatomy>]

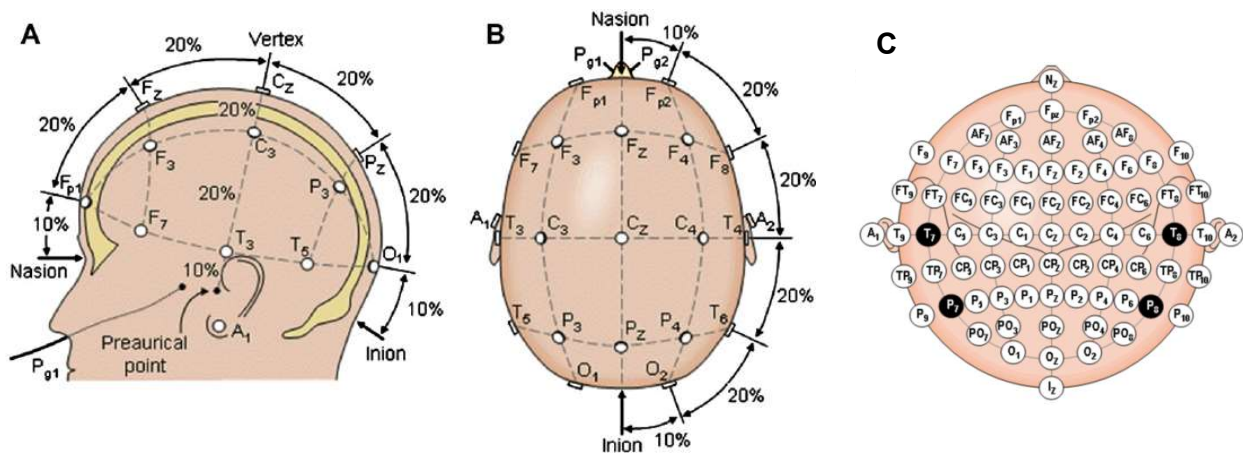
2.2.5.2. Importing EEG:

64-channel EEG data recorded in bio semi machine were imported directly into Brainstorm software in the form of. bdf file and the information content in raw file are

- ✓ sampling rate.
- ✓ Number of samples.
- ✓ Event markers and other details about the acquisition session.

2.2.5.3. Define channel location:

“The 10-20 electrode placement system is the standardized physical placement and designations of electrodes on the scalp. The head is divided into proportional distances from prominent skull landmarks (nasion, preauricular points, inion) to provide adequate coverage of all regions of the brain. Label 10-20 designates proportional distance in percent between ears and nose where points for electrodes are chosen. Electrode placements are labelled according to adjacent brain areas: F (frontal), C (central), T (temporal), P (posterior), and O (occipital). The letters are accompanied by odd numbers at the left side of the head and with even numbers on the right side.” [12]



“With the advent of multi-channel EEG hardware systems and the concurrent development of topographic methods and tomographic signal source localization methods, there was an increased need for extending the 10/20 system to higher density electrode settings. Therefore, the 10/10 system, an extension from the original 10/20 system with a higher channel density of 81, was accepted as a standard of the American Clinical Neurophysiology Society (ACNS; former American Electroencephalographic Society; Klem et al., 1999; American Electroencephalographic Society, 1994) and the International Federation of Clinical Neurophysiology (IFCN; former International Federation of Societies for Electroencephalography and Clinical Neurophysiology; Nuwer et al., 1998).” [12] See Fig. 2.4 above.

We imported Bio semi 10-10 system 64 electrode channel locations for our analysis which defines the types and names of channels that were recorded, the position of the sensors, the head shape and other various details.

2.2.5.4. Applying filters:

2.2.5.4.1. Notch Filter: To remove 60-Hz noise coming from the power line and their integral multiples, I used the inbuilt Notch filter from brainstorm toolbox.

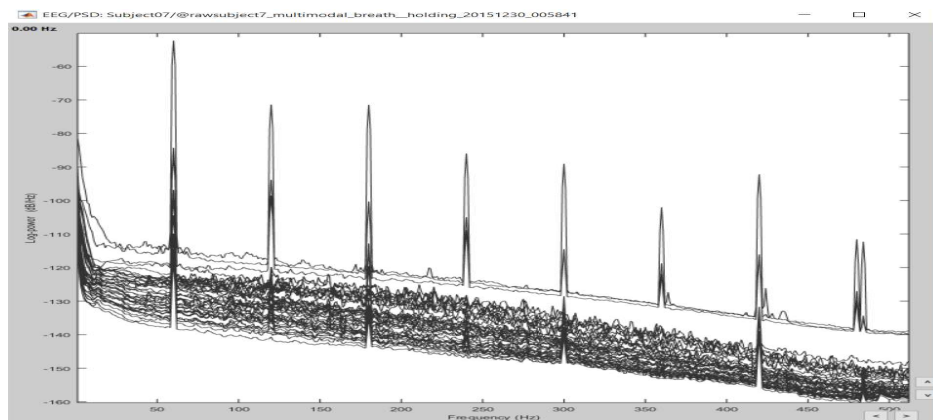


Fig 2.5: Need to removal of 60 Hz noise and its harmonic frequencies

2.2.5.4.2. Bandpass Filter: This filter removes any frequency band outside our area of interest which is 0-30 Hz. In this way, any high frequency noises (such as muscle noise) and low frequency noises <1 Hz (such as breathing or eye movement) as well as slow drifts of electrodes are all gotten rid of. A 2nd order

bandpass Butterworth filter was thereafter used to extract the specific EEG frequency bands. (See Fig. 2.6)

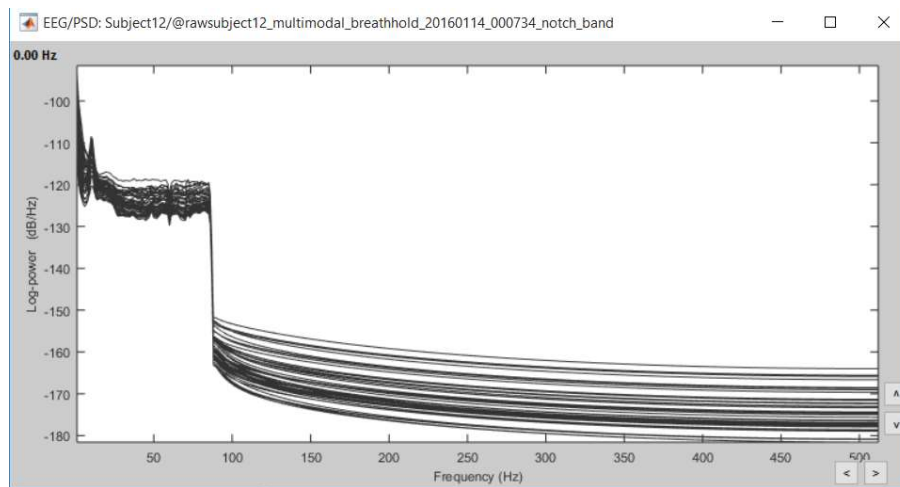


Fig 2.6: power spectrum of signal post Bandpass filter application

To estimate the EEG-based network connectivity among brain sites, the following three indices were calculated for the Delta band (0-4 Hz), theta band (4– 8 Hz), alpha band (8–12 Hz) and beta band (12–30 Hz).

2.2.5.5. ICA analysis:

The frequency filters are not adapted to remove artifacts that are transient or overlapping in frequency domain with the brain signals of interest. Because of the distance between the skull and brain and their different resistivity, EEG data collected from any point on the human scalp includes activity generated within a large brain area. “The joint problems of EEG source segregation, identification, and localization are very challenging, since the problem of determining brain electrical sources from potential patterns recorded on the scalp surface is mathematically underdetermined. By applying the ICA algorithm of Bell and Sejnowski, we attempt to separate the twin problems of source identification (What) and source localization (Where).” [13]

The ICA approach (Independent Component Analysis) relies on identifying spatial topographies that are specific from independent artifact/activities in time, such as line and muscle noise, eye movements, ECG, overlapping EEG phenomena, including alpha and theta bursts and spatially-separable ERP components. Then

we can remove these noise components from the recorded data set by subtracting them. Using the information from the time dimension makes it possible to work with a very low number of sensors, and to identify some components of the signal that are completely uncorrelated with the others.

The ICA technique appears ideally suited for performing source separation in domains where

- the sources are independent.
- the propagation delays of the 'mixing medium' are negligible.
- the sources are analog and p.d.f.'s not too unlike the gradient of a logistic sigmoid.
- the number of independent signal sources is the same as the number of sensors, meaning if we employ N sensors ($n=64$ in our case), the ICA algorithm results in N separate sources.

In the case of EEG signals, N scalp electrodes pick up correlated signals. We would like to know what effectively 'independent brain sources' generated these mixtures. We used Runica ICA algorithm in Brainstorm. [13]

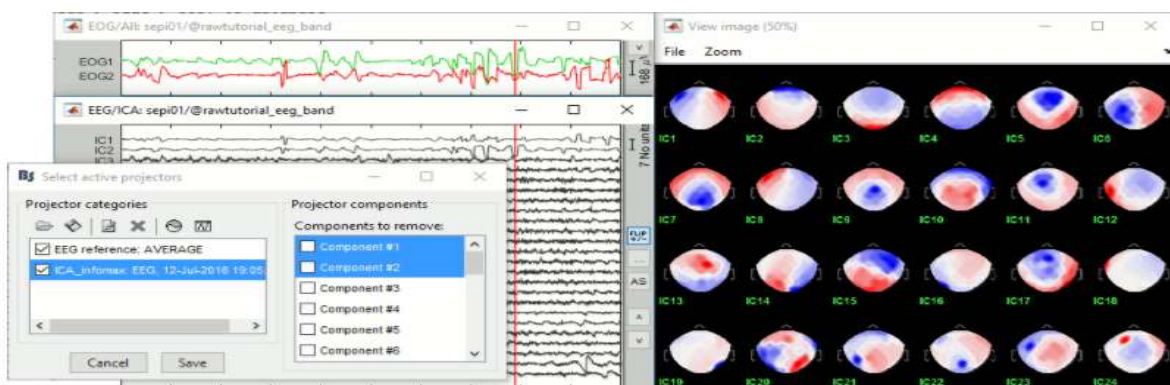


Fig 2.7: ICA analysis followed by removal of bad components

Fig. 2.7 shows 2D topography of 64 components corresponding to signals generated from 64 scalp electrodes. If the spatial topography of any artifact (such as line and muscle noise, eye movements, ECG) matches the time series of any of these components, that particular component is marked for removal. Consequently, the data will be made free of that known artifact.

2.2.5.6. Events classification:

Following the ICA analysis that allowed me to clean the data, I moved on to the events classification using Brainstorm as well. Out of the 10 minutes eyes closed data, all participants were marked for short periods of falling asleep at different sleep stages which were confirmed by a clinical sleep specialist based on several EEG channels of data. Specifically, the start time and duration of each stage of sleep were detected by the research team of our colleague, Professor Jae Gwan Kim from Biophotonics Lab (<http://biophotonics.gist.ac.kr>), School of Information & Communications, Department of Medical System Engineering, Gwangju Institute of Science and Technology (GIST), Gwangju, South Korea.

2.2.5.6.1. Sleep scoring by the experts:

Vigilance states were manually scored offline using 10-s epochs and were subdivided into active wake, NREMS and REMS as reported earlier. The waking state was identified by the presence of desynchronized EEG accompanied by a high EMG tone and/or muscle movement and eye movements in the EOG. NREMS was characterized by EEG synchronization (>75% epoch) and the appearance of spindle in EEG, no active muscle movement in EMG and reduced eye movements in EOG. REMS were identified using EEG desynchronization, muscle atonia and frequent eye movements, usually following NREMS. Based on the physiological sleep profile the representative collections of 10 sec epochs (n = 240 for each stage) of the wake, NREMS and REMS stages were subjected to programs written in MATLAB for further analysis.

Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Resting(Eyes_open)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Resting(Eyes_closed)	10	9.5	8.5	10	5	10	5.5	1.5	1	9.5	2	5.5	0	3	0.5	10	10	0.5
Sleeping_Stage 1	0	0.5	1.5	0	5	0	4.5	4.5	4	0.5	4	4.5	4	1.5	4	0	0	1.5
Sleeping_stage 2	0	0	0	0	0	0	0	4	5	0	4	0	6	5.5	5.5	0	0	8

Table T1: Sleep scoring and their time duration at each stage

The above chart shows the time duration of each of 18 participants in each stage of sleep as analyzed by the sleep expert in GIST, Korea. Based on the table, each

subject's data in all four bands (i.e., Delta, Theta, Alpha and Beta) were event classified into Eyes open, Eyes closed, Sleep stage 1, Sleep stage 2 (See Fig. 2.8).

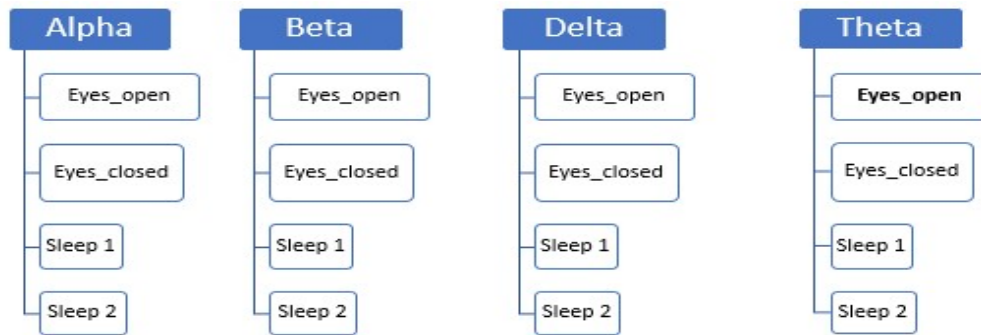


Fig 2.8: Classification of different vigilance states across four frequency bands

Different colors across four rows (i.e., four states: eyes-open, eyes-closed, sleep stage 1, and sleep stage 2) in Table T1 signify which subjects were taken into consideration for which stages with how many minutes. Then such chosen time-dependent data were used to calculate correlation matrices among each pair of electrodes for each of the four states in all four bands. It is seen that not all subjects went through all four stages for a definite time length. Also, some of the subjects' data after ICA analysis were still too noisy to be analyzed further, so I had to exclude them. This was why the total numbers of subjects used for data processing at each state were not the same in further analysis.

Overall, given all the factors and considerations mentioned above, I made my specific selections for my EEG data analysis. The data selections are summarized below:

- **Resting eyes-open state (EO):** 10 subjects with a 5-min recording time.
- **Resting eyes-closed state (EC):** 7 subjects with a 3-min or longer recording time.
- **Sleep stage 1 (SS1):** 7 subjects with a 4-min or longer recording time.
- **Sleep stage 2 (SS2):** 6 subjects with a 4-min or longer recording time.

2.2.6. Correlation:

A correlation method was used to measure brain network connectivity in my study. There is no gold standard method to perform/quantify EEG brain connectivity. Every method is known to have its pros and cons. Fig. 2.9 is a chart to present features of different existing connectivity methods. [14]

Table 1
The properties of the different described functional connectivity measures.

	Undirec	Direc	Bivar	Multivar	Ampl	Phase	Lin	Nonlin	Time	Freq
Correlation	X		X		X		X		X	
Coherency		X	X		X		X			X
Cross-correlation		X	X		X		X		X	
Directed coherence		X		X	X		X			X
Directed transfer function		X		X	X		X			X
Granger causality index		X	X		X		X		X	
Mutual information	X		X		X			X	X	
Partial coherence	X			X	X		X			X
Partial directed coherence		X		X	X		X			X
Phase locking value		X	X			X		X	X	
Phase lag index		X	X			X		X	X	
Transfer entropy		X	X		X			X	X	

Abbreviations: Undirec = undirected, Direc = directed, Bivar = bivariate, Multivar = multivariate, Ampl = amplitude, Lin = linear, Nonlin = nonlinear, Freq = frequency.

Fig 2.9: Different connectivity methods and their features

2.2.6.1. What is correlation?

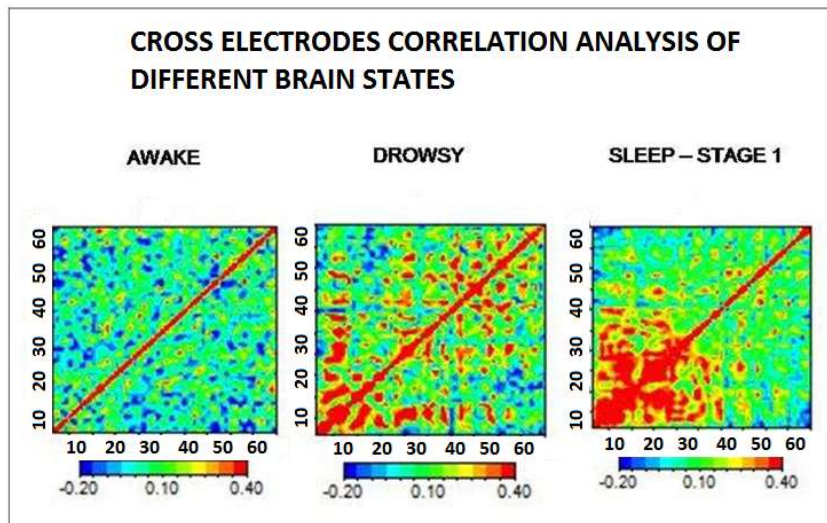


Fig 2.10. Example of power correlations from 1 to 64 electrodes of the same subject at different brain states, i.e. wakefulness, drowsy and sleep.

“In general, correlation may be defined as the linear relation between the amplitudes of two signals. It is a common method of evaluating bi-spectral relations to compute the cross-electrode power correlation (Linás et al, 2005). The method involves computing the covariance of power at each electrode with respect to all other electrode locations.” [15] Fig. 2.10 above shows an example.

2.2.6.2 Pros:

- It is commonly used;
- It is a straightforward method.

2.2.6.3 Cons:

- Nonlinearity is not considered.
- It is not possible to make a distinction between direct and indirect relations.
- It is sensitive to volume conduction.

I performed $N*N$ correlation ($N=64$) for all the events of all the subjects across all bands. Since EEG is not an uncorrelated stochastic signal, there always exists an intrinsic temporal structure. As a result, the sampling rate will affect the result of the correlation analysis, e.g., under sampling will underestimate the correlation whereas oversampling will exaggerate the correlation. Such a bias can be avoided by complying the sample rate with the frequency of the signal. But since EEG is non-stationary and its dominant frequency varies from state to state, it is recommended to treat all EEG components. Thus, I separated EEG temporal rhythms into delta, theta, alpha and beta frequency bands, followed by correlation analyses across all four bands instead of a broadband (0-30 Hz). [16]

2.2.7. Statistical Analysis:

All the statistical analyses were performed using Microsoft excel and MATLAB. Several steps were taken to (1) obtain the correlation graphs using Brainstorm first, (2) calculate Z values by the Fisher transformation, and then (3) perform Student T-test for comparison of each pair of two states (i.e., EO vs. EC, EC vs. SS1, and SS1 vs. SS2). See the following sub-sections for details.

2.2.7.2. Explanation of the steps shown in 2.2.7.1:

- A- A 64*64 correlation graph of an awake/sleep state (e.g., 'EO' state) of any one of the subjects for a certain band; completed using Brainstorm.
- B- The corresponding correlation graph represented in the form of a matrix.
- C- If a diagonal is drawn across the correlation matrix from B, the upper half is nothing but a mirror image of the lower half of the matrix. Now this matrix cannot be exported to MATLAB if keeping only half of the matrix.

- Original matrix size $64*64=4096$
- When being exported to MATLAB, 64 is subtracted at first from the total number of elements to remove all the 1's along the diagonal line and then divided by 2 to make it a half triangle. Then, the total number of elements becomes

$$\frac{(64*64)-64}{2}=2016$$

- D- The correlation matrix is converted in the form of half triangle (right angle triangle).
- E- The initially subtracted 64 elements are now added back to the half matrix to form a column vector of size $2080*1$ ($2016+64=2080$), which is now ready to be exported to MATLAB. This is an example to represent an EO resting state of one subject at one frequency band.
- F- For " $n=10$ " subjects at the EO resting state with one frequency band, I have a correlation matrix of $2080 \times n$.
- G- To perform the Fisher transformation for the above matrix, correspondingly, I obtain a Z matrix with a matrix size of $2080 \times n$.
- H- Similarly, another Z matrix for " $k=7$ " subjects, at the EC resting state with the same frequency band can be obtained with a matrix size $2080 \times k$. Now I can compare these two Z matrices between EO and EC to calculate/achieve a T-value column vector of the size $2080*1$.
- I- This is a T-value column of size 2080×1 from two Z-matrix comparisons.
- J- Conversion from the T-value column of 2080×1 to a square T-value matrix of 64×64 to identify locations/channels that have significant changes in correlations between pairs of electrodes for the chosen two states (e.g., EO vs. EC).

Since I looked at four vigilance states, there were 3 time sequences:

- Eyes open Vs Eyes closed
- Eyes closed Vs Sleep stage 1
- Sleep stage 1 Vs Sleep stage 2.

Also, I looked at four frequency bands (namely delta, theta, alpha and beta) for each of three comparisons. So, I had $4*3=12$ comparisons in total, namely, 12 T-matrices were obtained with a matrix size of $64*64$.

2.2.7.3. T-test:

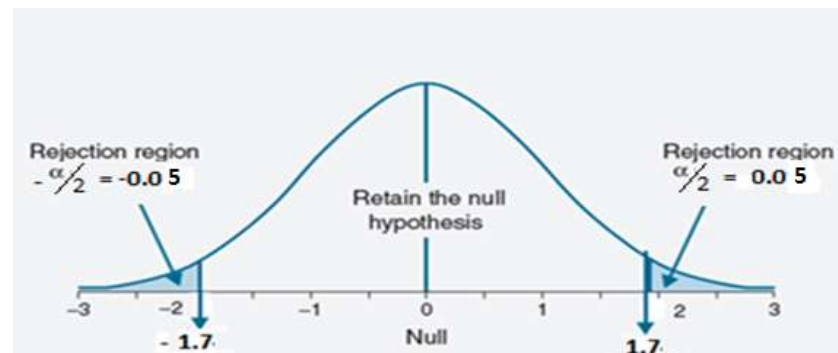


Fig 2.11: T-test bell curve

Each of the T-matrices is spread across the bell curve. Since I was only interested in finding the T-values that cause significant changes in brain connectivity at a certain state, I performed a two-sample T-test and considered the values that were lying in the rejection region.

So, the test hypotheses can be formed as follows-

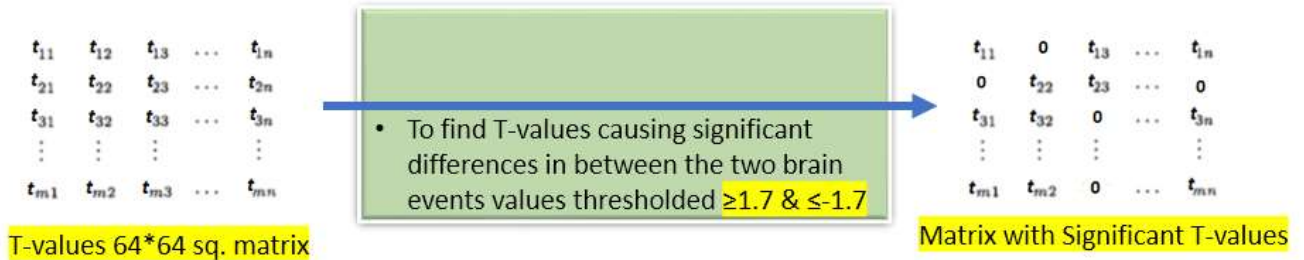
Null hypothesis:

$H_0: \mu_1 = \mu_2$ i.e. there is no significant differences in brain state in between two comparisons.

Alternate hypothesis:

$H_a: \mu_1 \neq \mu_2$ i.e. there are significant differences in brain state in between two comparisons.

With the degree of freedom 34, significance level 0.1, my critical T-score value was 1.69 which was rounded up to 1.7. So, all the T-matrices were thresholded to keep only the T values larger than 1.7 and smaller than <-1.7 and all other insignificant values were set to be zero, as demonstrated below.



2.2.8. Colormap plot:

The new T-matrices with significant values are plotted in a colormap using MATLAB to show the locations of changes taking place. In the following (Fig. 2.12) is an example of colormap plot of significant T-matrix between eyes closed (EC) vs. sleep stage 1 (SS1) for the alpha band, where the regions with light green color signifies regions with positive T-values over 1.7 and dark blue regions signifies locations having T-values less than -1.7. The regions with light blue color mark the brain/cortical locations that do not have much significant changes in brain connectivity when the brain switches from one stage to another. The two black lines are across at a green spot, meaning that the brain connectivity between these two electrode locations is altered or changed when the brain is switched from EC to SS1. Specifically, the point location of green region corresponds to electrode locations of AFz and Fz, meaning the brain network connectivity is in the antero-frontal region.

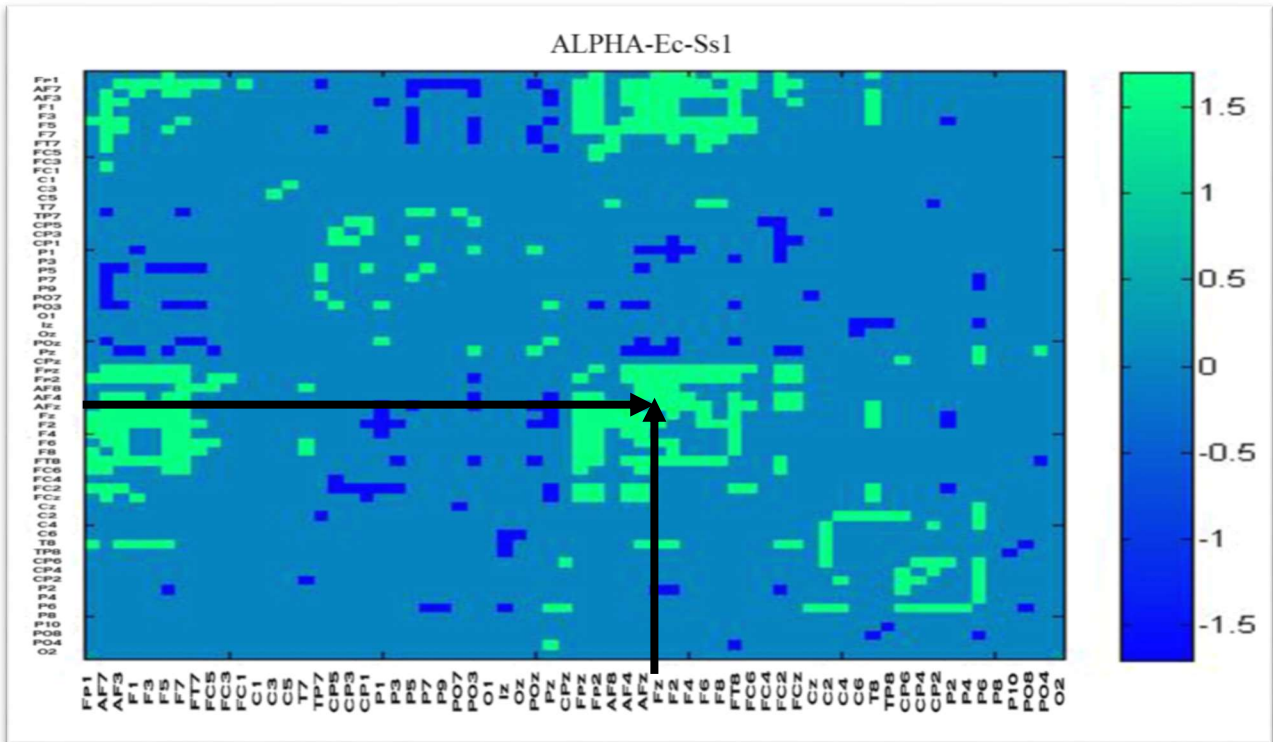


Fig 2.12: Colormap plot of Eyes closed vs SS1 over the Alpha band

2.2.9. Functional connectivity:

To analyze functional connectivity between all pairs of electrodes, I modified an inbuilt function, `topoplot`, which is part of EEGLAB in MATLAB. It was a method for evaluating the similarity between signals in the frequency domain.

Usage and inputs:

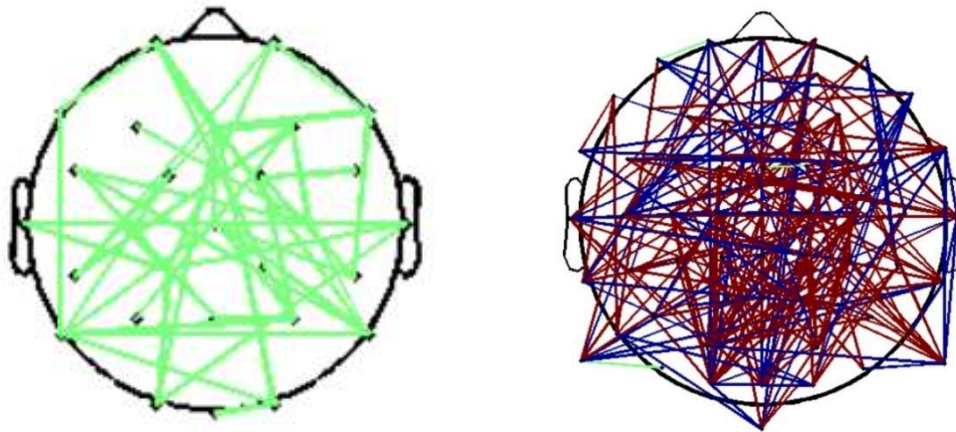
```
>>topoplot_connect (ds, EEG.chanlocs);
```

ds is the display structure with the following fields:

- `ds.chanPairs`- N x 2 matrix, with N being the number of connected channel pairs. Given 64 channels since we have created connection of each channel with every other 63 channels, total no of connected channel pairs is 64*63

- `ds.connectStrength*` - $N \times 1$ matrix, a vector specifying connection strengths. The measure of connectivity between channel pairs is obtained from the T-value matrix. Since we are only interested in color coding the connection strengths of significant connections, we assigned red color to the values above 1.7 and blue to the ones below 1.7.

`EEG.chanlocs` is a structure specifying channel locations (or an `locs` filename).



Connected topo plot

With connection strengths

Fig 2.13: Topo plot of connection strengths.

[\[https://praneethnamburi.wordpress.com/2011/08/18/connectedtoplot/\]](https://praneethnamburi.wordpress.com/2011/08/18/connectedtoplot/)

In my analysis, I mainly looked at four different plots for each of the three comparisons eyes open Vs eyes closed, eyes closed Vs sleep stage 1 and sleep stage 1 Vs sleep stage 2. They are:

- Pooled connectivity plot for all four bands.
- Bar plot to show the number of connectivity across all four bands.
- Electrode based topography for in depth analysis of each of 64 electrodes.
- Electrodes showing maximum connections during each state.

CHAPTER 3

RESULTS, OBSERVATION AND SPECULATION

3.1. Comparison 1: Eyes open Vs Eyes closed

3.1.1. Pooled connectivity plot

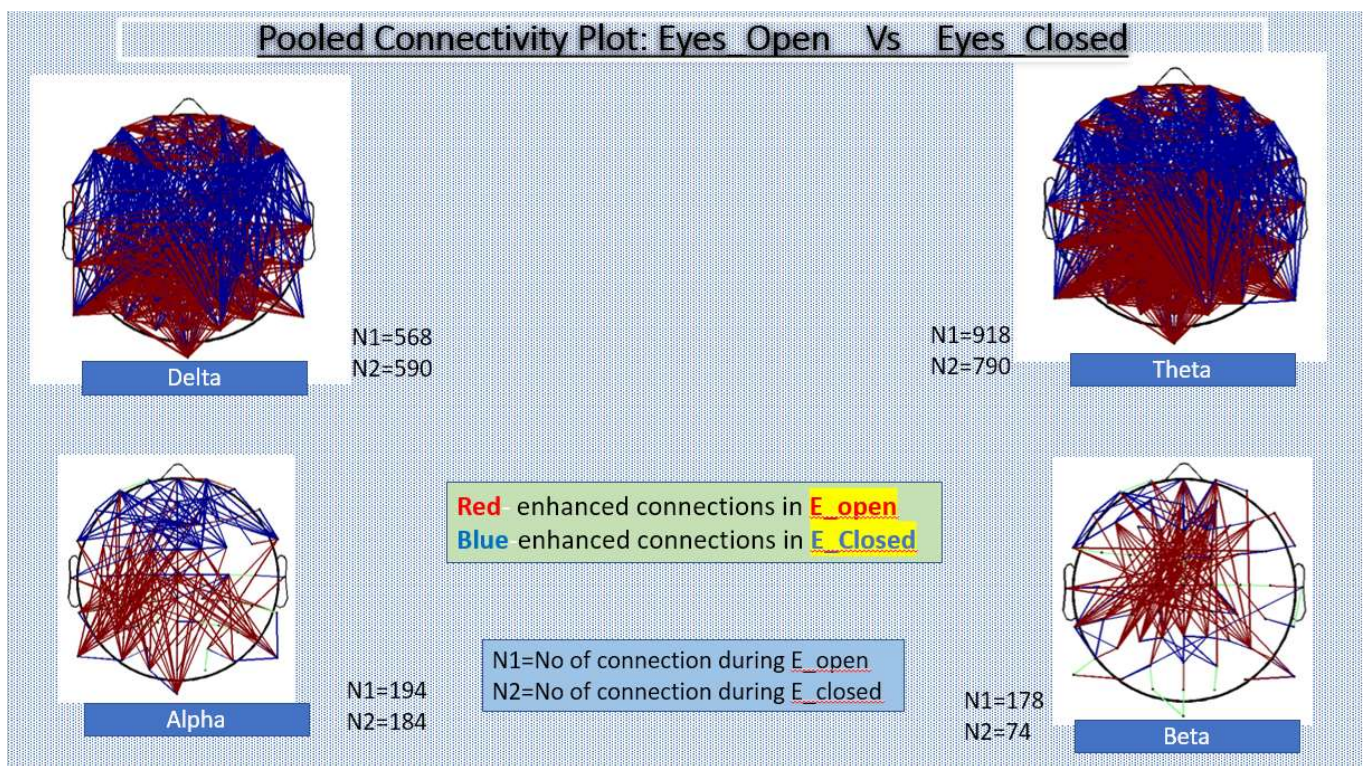


Fig 3.1: Pooled connectivity plot of eyes open Vs eyes closed

3.1.1.1. Observation:

The red region in Fig. 3.1 shows significantly enhanced connections in eyes open state compared to eyes closed. The blue region shows significantly enhanced connections in eyes closed state compared to eyes open.

The number of enhanced connections varies in all four bands. N1 and N2 mark the number of significant connections during eyes open and eyes closed state, respectively, across all four bands (delta, theta, alpha and beta).

- Major connectivity alteration observed in Delta & Theta band. During eyes open red regions are more concentrated in left parietal-occipital, whereas during eyes closed state the same concentration is noticed in central parietal region.
- Not much change is observed in theta band compared to delta, only number of enhanced connections improved in theta band where we see significant connections during eyes open state went up from 568 to 918 and there is also increase in connections during eyes closed from 590 to 790.
- Alpha & beta does not show much significant changes. Alpha shows more localized connections than long distance/across connections with eyes open connections (red) more towards the posterior part and eyes closed connections (blue) more on the anterior part of the brain.
- Beta band shows almost no connections during eyes closed state.

3.1.1.2. Speculation:

- Since alpha band lies in the frequency range 8-12 Hz, it is expected to show more connections during eyes open state when brain is processing more visual information and delta band (0-4 Hz) is more active during eyes closed when the brain is processing comparatively less information flow due to absence on visual information intake. Now theta band (4-8 Hz) is an intermediate band between delta and alpha, meaning the connections in this band mostly owes to a state in between eyes open and eyes closed. Delta being more prone to eyes closed connections is considered to be

more idle state compared to theta. So more idle state meaning brain going thru less activity, less connections. That is why delta has less connections than theta band in both eyes open and eyes closed.

- “The front to back connectivity during eyes closed is due to exchange of strong information in between the alpha and theta band, which form a loop through which information “reverberates” or circulates. A pattern of anterior-to-posterior flow was found in the theta band, involving mainly regions in the frontal lobe that were sending information to a more distributed network. Interestingly, strong senders of information in the alpha band were also often receivers of information in the theta band, and vice versa, suggesting a frequency-specific loop of information flow in the human brain”. [17]
- During eyes open, there is a continuous flow of visual information from front to back which is discontinued when eyes are closed. Therefore, power flow from anterior to posterior during eyes open is cut off in the middle during eyes closed due to discontinue of information flow which is then stuck at back causing more enhanced dense connections in front. This dense connection area is better known as anterior default mode network prominent during eyes closed state.
- Beta is a high frequency band compared to alpha. Since the transition is taking place from eyes open to eyes closed state, connections are expected to go down in higher frequency bands due to less brain activity and more concentrated in lower frequency bands such as delta and theta which is why beta band shows practically no connections in eyes closed condition.

3.1.2. Bar plot: Eyes open vs eyes closed

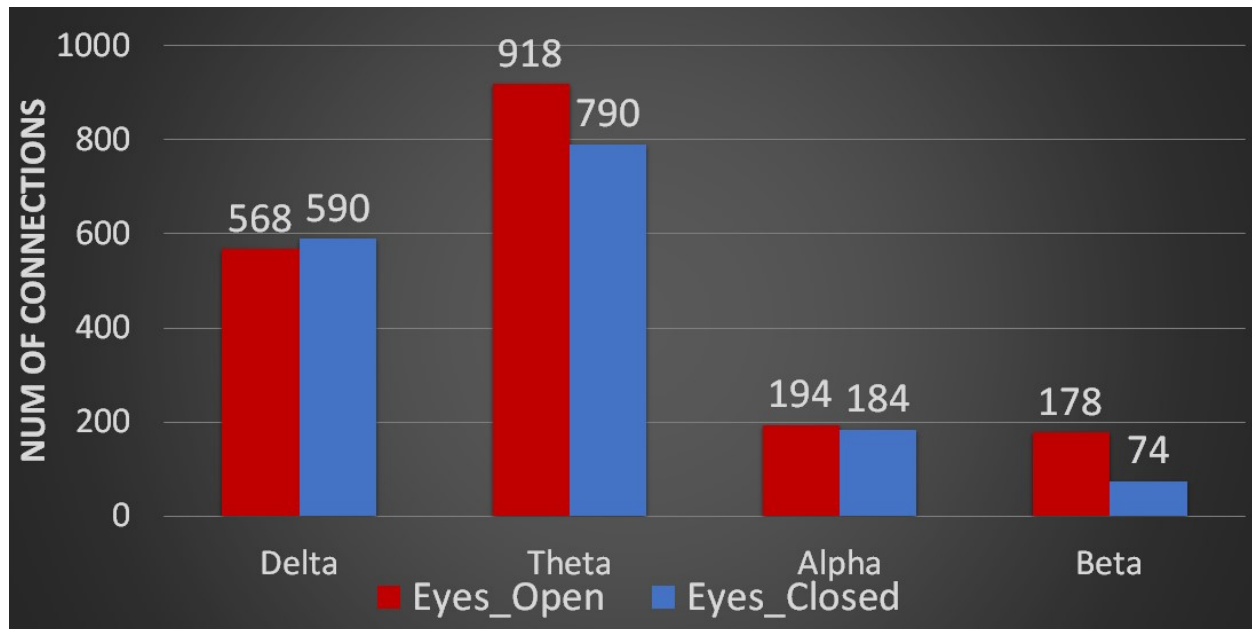


Fig 3.2: Bar plot showing number of connectivity in each state eyes open and eyes closed

3.1.2.1. Observation:

The red and blue bars correspond to number of significant connections during eyes open and eyes closed respectively across all four bands-delta, theta, alpha and beta.

- Theta band shows more significant connections than delta as we can see from the above barplot which is in accordance to pooled connectivity plot.
- Overall eyes open connections are higher in number compared to eyes closed, signifying there is a decrease in brain activity when a person closes his/her eyes. The loss of visual information due to eyes being closed explains the cause.

3.1.3. Electrode Based topography:

3.1.3.a. Delta band

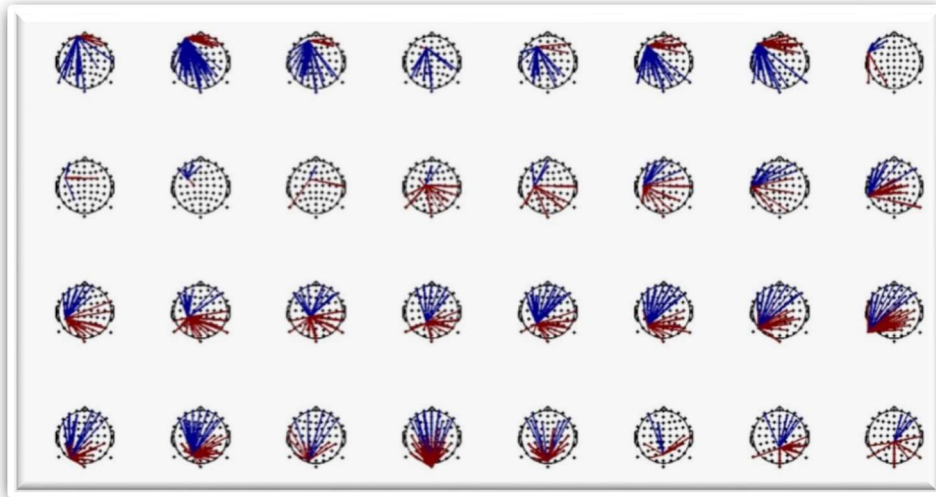


Fig 3.3: Electrode based topography of 1-32 electrodes in delta band

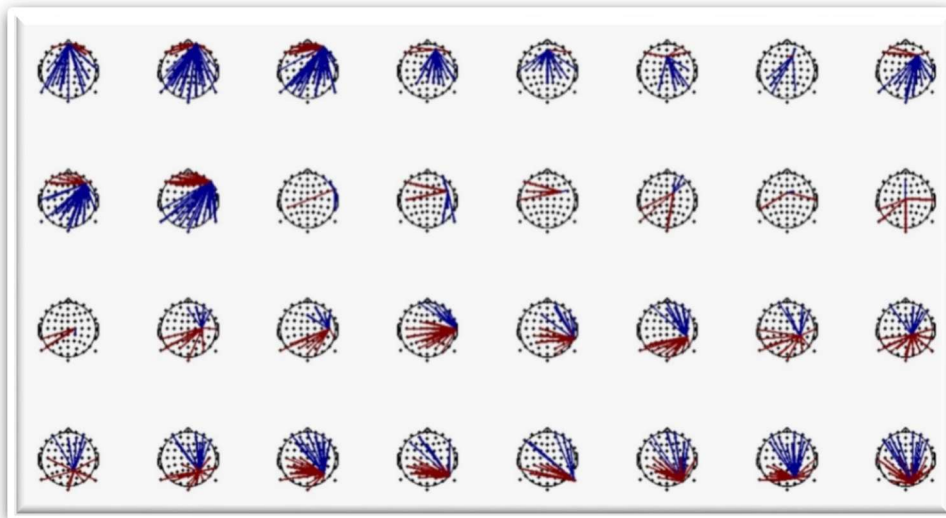


Fig 3.4: Electrode based topography of 33-64 electrodes in delta band

3.1.3.b. Theta band

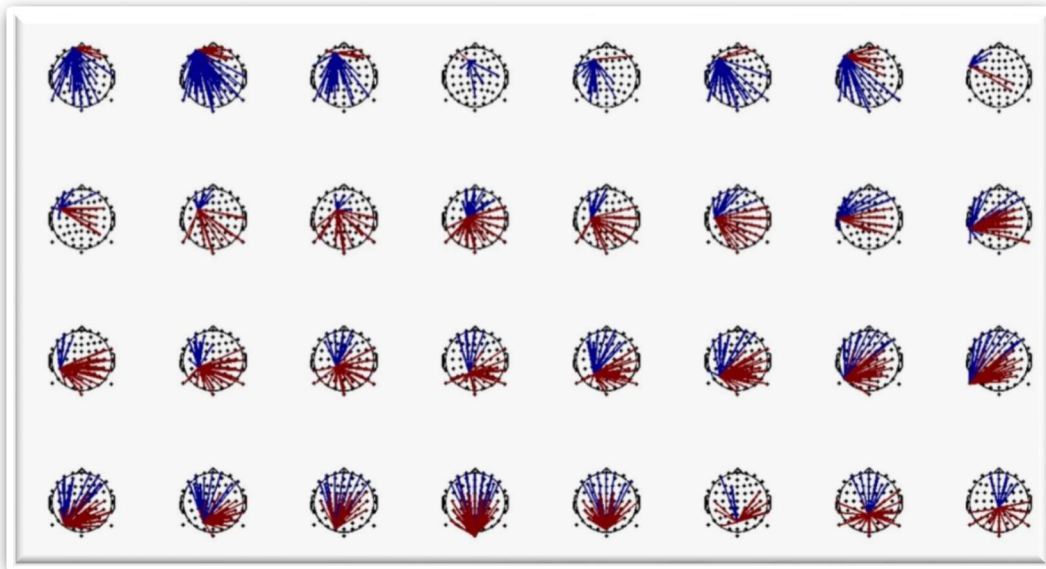


Fig 3.5: Electrode based topography of 1-32 electrodes in Theta band

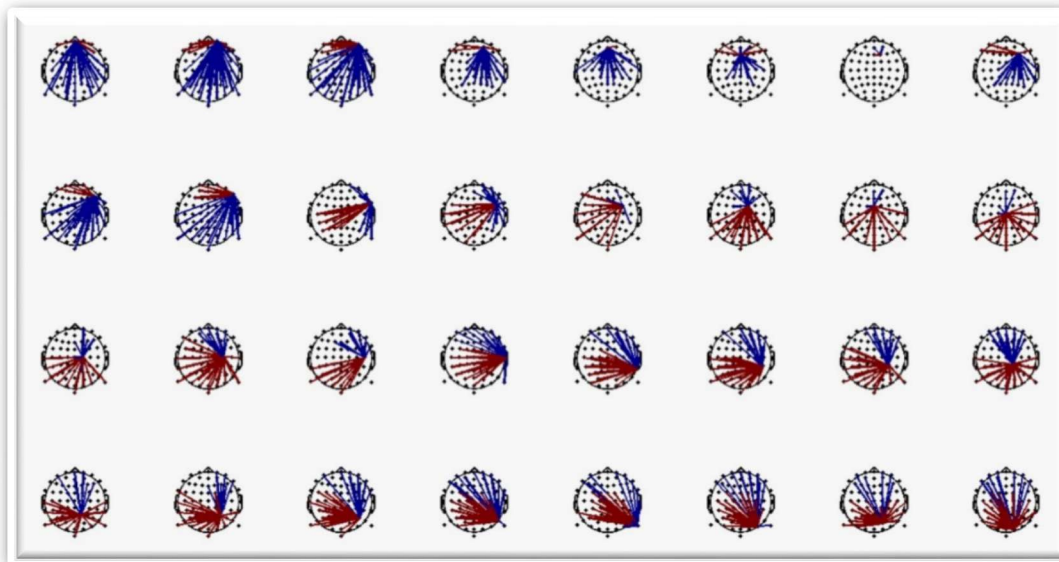


Fig 3.6: Electrode based topography of 33-64 electrodes in Theta band

3.1.3.1. Observation:

- The red and blue lines in Figs. 3.3-3.6 correspond to significant connections across all 64 electrodes during eyes open and eyes closed respectively. For delta, strength of connections during eyes closed are
 - ✓ Mostly long distance based across anterior to posterior.
 - ✓ Decreasing interhemispheric-ally, being localized in either anterior or posterior.
- For theta, in addition to delta features,
 - ✓ Motor sensory area (first three rows in Fig. 3.5) showing more connectivity change than delta during eyes open.

3.1.3.2. Speculation:

- When we see something, these visual information goes first from frontal to occipital and then to motor-sensory part giving rise to increase in connectivity in that region in theta band. Sensory cortex is related to auditory, visual, olfactory, somatosensory cortex and motor cortex helps in execution of voluntary movements. These activity is disappeared during delta region when eyes are closed resulting from the discontinue of information from frontal region. As the information cannot reach the motor sensory region we see significant reduction in connections in that region in delta band during eyes closed.

3.1.4. Electrodes showing maximum connections in each state across all four bands:

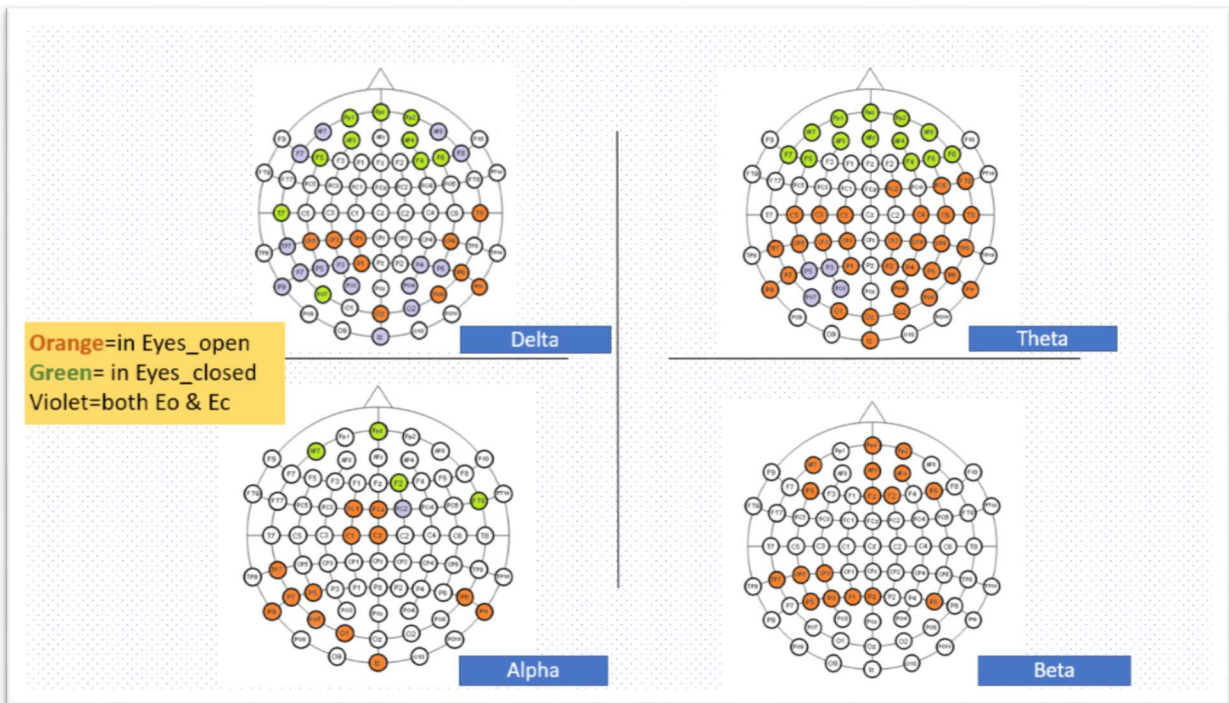


Fig 3.7: Electrodes showing maximum connections in Eyes_open & Eyes_closed across all four bands

3.1.4.1. Observation:

This is a 2d plot of the entire head to show which electrodes contribute most to significant changes during eyes open and eyes closed state across different bands.

Orange and green colored electrodes show major connectivity change during eyes open and eyes closed states respectively. And electrodes marked violet show significant changes in both the states.

- More number of electrodes in theta band contributing to significant changes compared to delta.
- Alpha shows more electrodes contributing to eyes closed connections in anterior part and eyes open connections denser at the posterior part.

3.2. Comparison 2: Eyes closed Vs Sleep stage 1

3.2.1. Pooled connectivity plot:

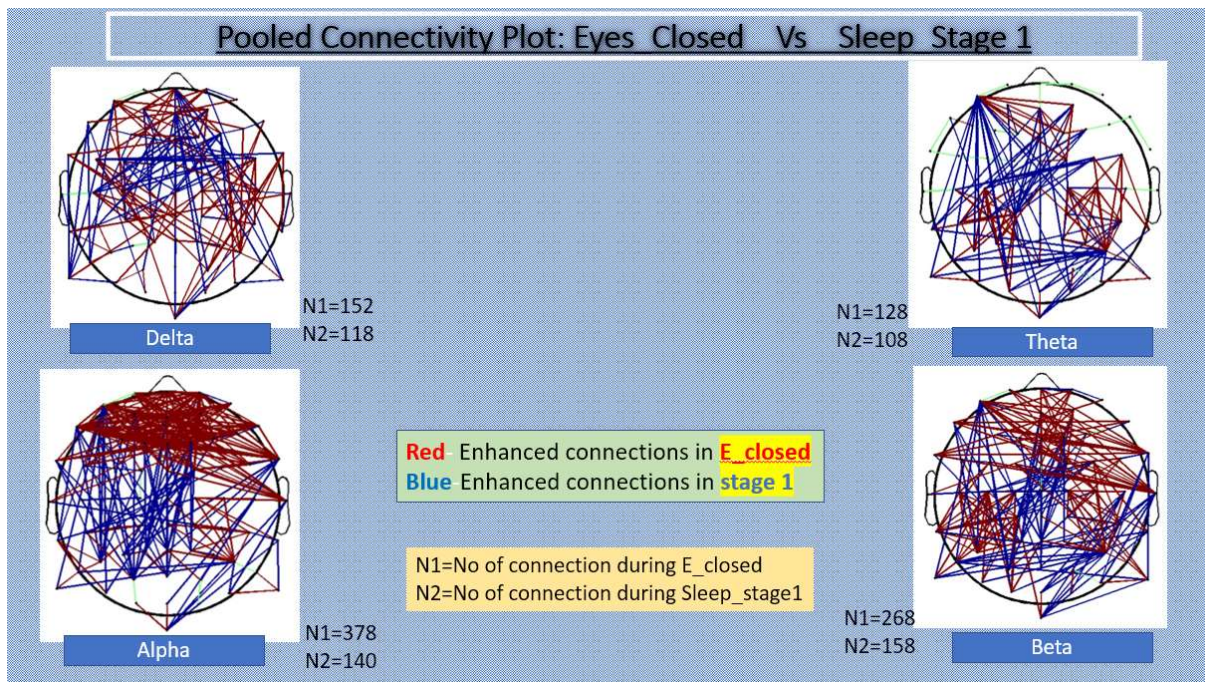


Fig 3.8: Pooled connectivity plot of eyes closed Vs sleep stage 1

3.2.1.1. Observation:

The red region in Fig. 3.8 shows the enhanced connections in eyes closed state compared to sleep stage 1. The blue region shows the enhanced connections in sleep stage 1 compared to eyes closed.

The number of enhanced connections varies in all four bands. N1 and N2 show the number of significant connections during eyes closed and sleep stage 1 respectively across all four bands delta, theta, alpha and beta.

- More connectivity change in Alpha band, especially Frontal Alpha where the connections (red region) are denser during eyes closed which disappears during sleep stage 1 and concentration shifts towards central parietal region.

3.2.1.2. Speculation:

During eyes closed state, there is no visual information flow from front to back resulting in the dense connectivity localized only in frontal part, which is also known as anterior default mode network. So, the result here is in accord with previous studies saying anterior default mode network is prominent in eyes closed resting state. This anterior default mode network is expected to disappear in sleeping stages which is visible from the plot where we see during sleep stage 1 the dense connections from the anterior frontal region is shifted more towards the central parietal (blue region) as the subject gradually goes from 'just eyes closed' state to first stage of sleep.

3.2.2. Bar plot: Eyes closed vs Sleep stage 1

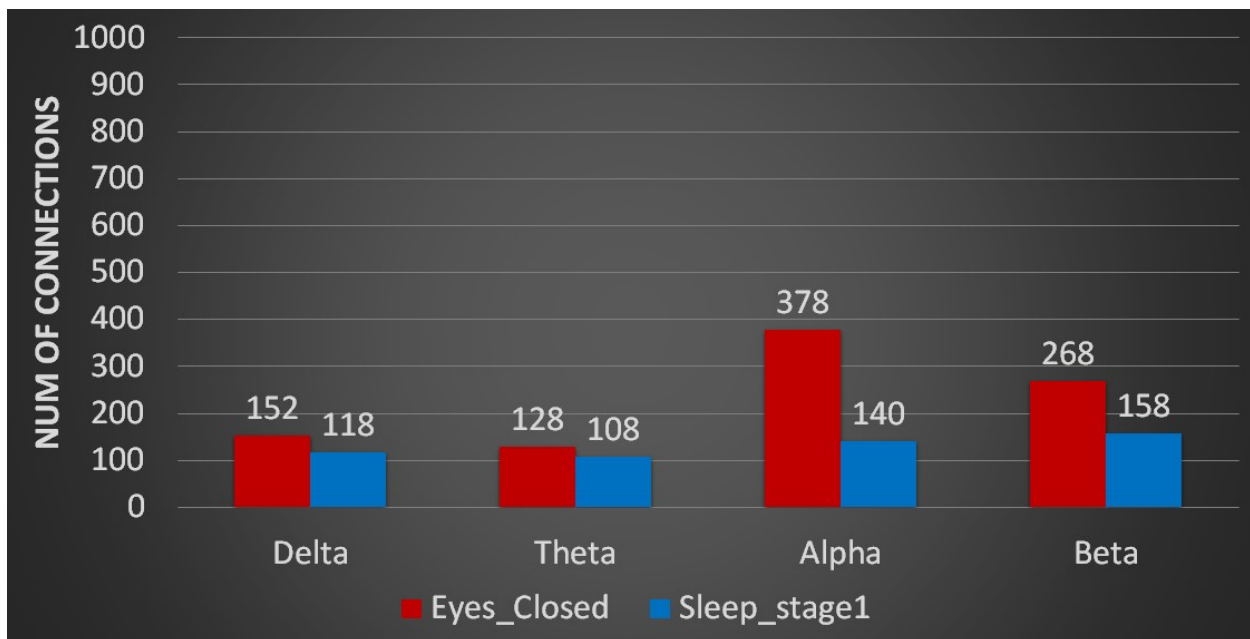


Fig 3.9: Bar plot showing number of connectivity in each state eyes closed and sleep stage 1

3.2.2.1. Observation:

The red and blue bars correspond to number of significant connections during eyes closed and sleep stage 1 respectively across all four bands-delta, theta, alpha and beta.

- Alpha band shows most number of significant connections in eyes closed state (number of significant connections 378) as we can see from the above barplot which is in accordance to pooled connectivity plot.
- Overall, more number of connections during eyes closed state compared to sleep stage 1. Sleep stage 1 is more idle stage compared to eyes closed. So, less brain activity in sleep stage 1 leads to less connections.

3.2.3. Electrode Based topography:

3.2.3.a. Alpha band

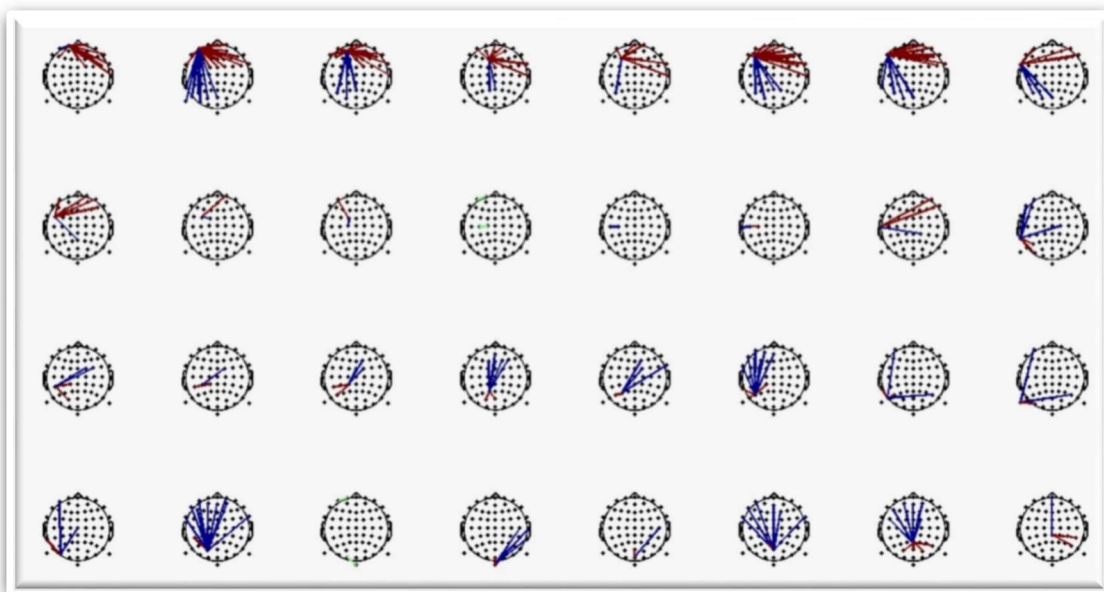


Fig 3.10: Electrode based topography of 1-32 electrodes in alpha band

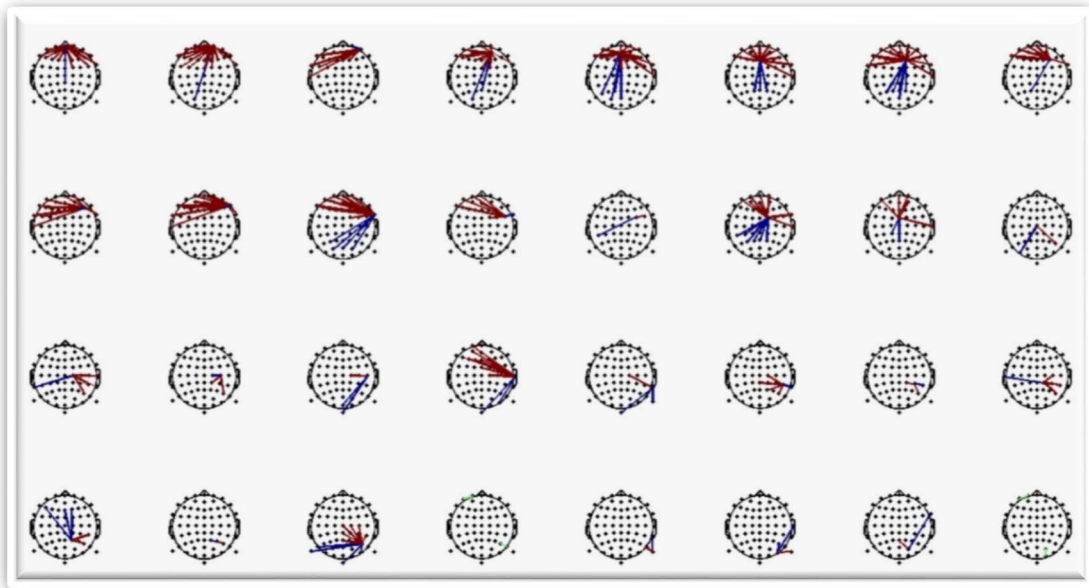


Fig 3.11: Electrode based topography of 33-64 electrodes in alpha band

3.2.3.1. Observation:

The red and blue lines correspond to significant connections across all 64 electrodes during eyes closed and sleep stage 1 respectively.

- The dense connectivity (red region) during eyes closed state only in frontal region in alpha band across most of the electrodes suggests the connections are more localized instead of long distance anterior to posterior, which is in accordance with previous plots.
- The emergence of blue region signifying major connection changes across sleep stage 1 can also be considered as loss of red regions meaning connections during sleep stage 1 significantly disappears from anterior frontal part (default mode network) and decreases compared to eyes closed due to loss of consciousness during sleep.

3.2.4. Electrodes showing maximum connections in Eyes closed & Sleep-stage1

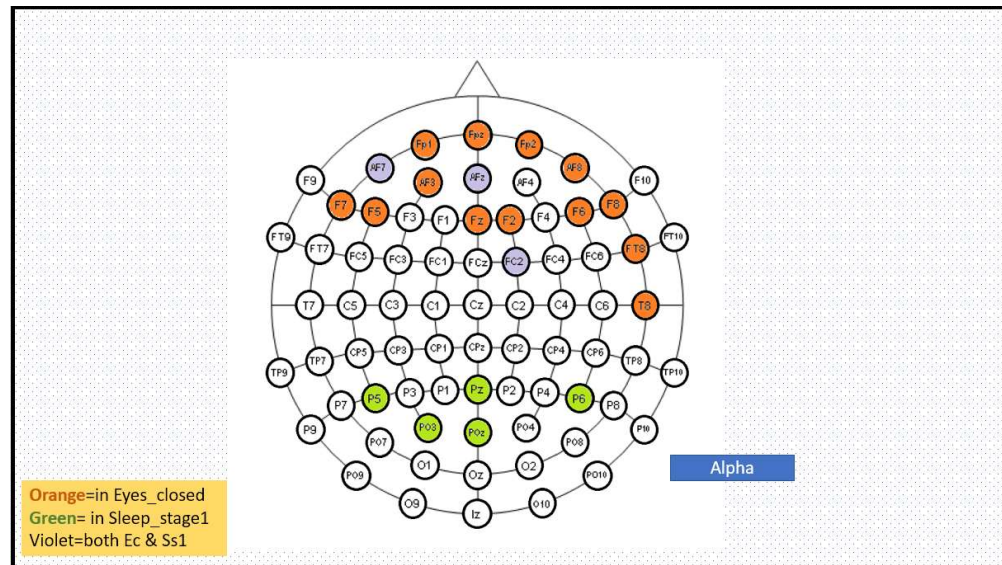


Fig 3.12: Electrodes showing maximum connections in Eyes closed & Sleep-stage1

3.2.4.1. Observation:

This is a 2d plot of the entire head to show which electrodes contribute most to significant changes during eyes closed and sleep stage 1 across different bands.

Orange and green colored electrodes show major connectivity change during eyes closed and sleep stage 1 respectively. And electrodes marked violet show significant changes in both the states.

- Alpha band shows more electrodes contributing to formation of anterior default mode network during eyes closed connections and only a handful of electrodes contributing to major connections during sleep stage 1 in central parietal region.

3.3. Comparison 3: Sleep stage 1 Vs Sleep stage 2

3.3.1. Pooled connectivity plot:

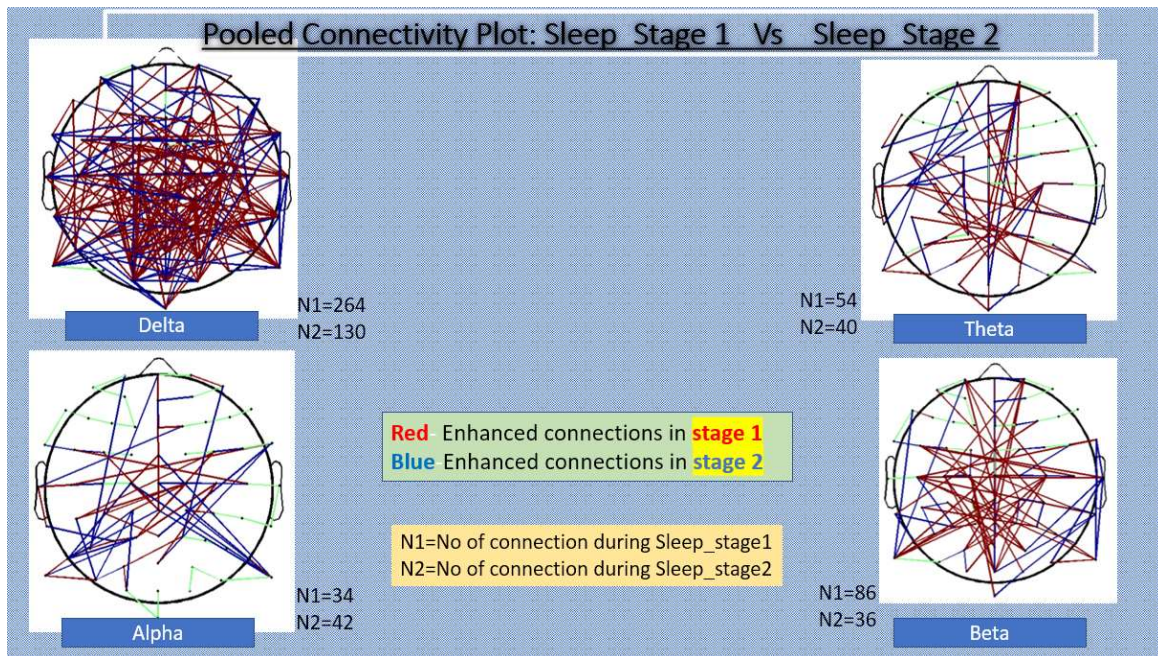


Fig 3.13: Pooled connectivity plot of sleep stage 1 vs sleep stage 2

3.3.1.1. Observation:

The red region shows the enhanced connections in sleep stage 1 compared to sleep stage 2. The blue region shows the enhanced connections in sleep stage 2 compared to sleep stage 1.

The number of enhanced connections vary in all four bands. N1 and N2 shows the number of significant connections during sleep stage 1 and sleep stage 2 respectively across all four bands delta, theta, alpha and beta.

- Major connectivity alteration in Delta band, which is consistent with general knowledge from previous studies.
- All other bands are expected to show less or almost no significant connections.

3.3.1.2. Speculation:

Delta is major dominant during deep sleep. Since sleep is an extreme idle state and we are apparently in unconscious state experienced by the loss of physical awareness, brain activity is very less and is specifically prevalent in delta band which has a low frequency range 0-4 Hz. Due to very less brain activity, delta band is associated with the deep stage of NREM sleep, also known as slow-wave sleep (SWS), and aid in characterizing the depth of sleep.

3.3.2. Bar plot: sleep stage 1 Vs sleep stage 1

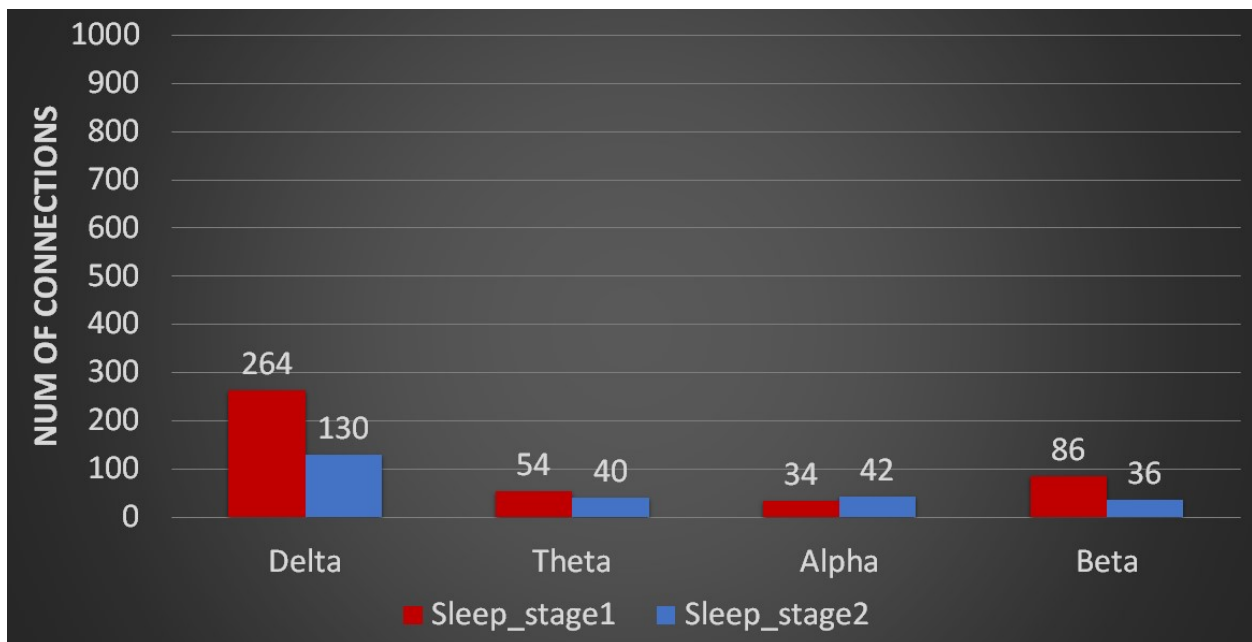


Fig 3.14: Bar plot showing number of connectivity in each state sleep stage 1 and sleep stage 1

3.3.2.1. Observation:

The red and blue bars correspond to number of significant connections during sleep stage 1 and sleep stage 2 respectively across all four bands-delta, theta, alpha and beta.

- The number of connections during sleep stage 1 (264) is almost double of connections in sleep stage 2 (130).

3.3.2.2. Speculation:

- Going into deeper sleep due to absolute unconscious state we see very low activity in brain, resulting in significant drop in number of connections from stage 1 to stage 2. Sleep stage 1 is less idle, more of a 12 am sleep compared to sleep stage 2 which we have at 3 am at night when we might have already seen 2 or 3 dreams. More idle state leads to lesser connections.

3.3.3. Electrode Based Topography:

3.3.3.a. Delta band

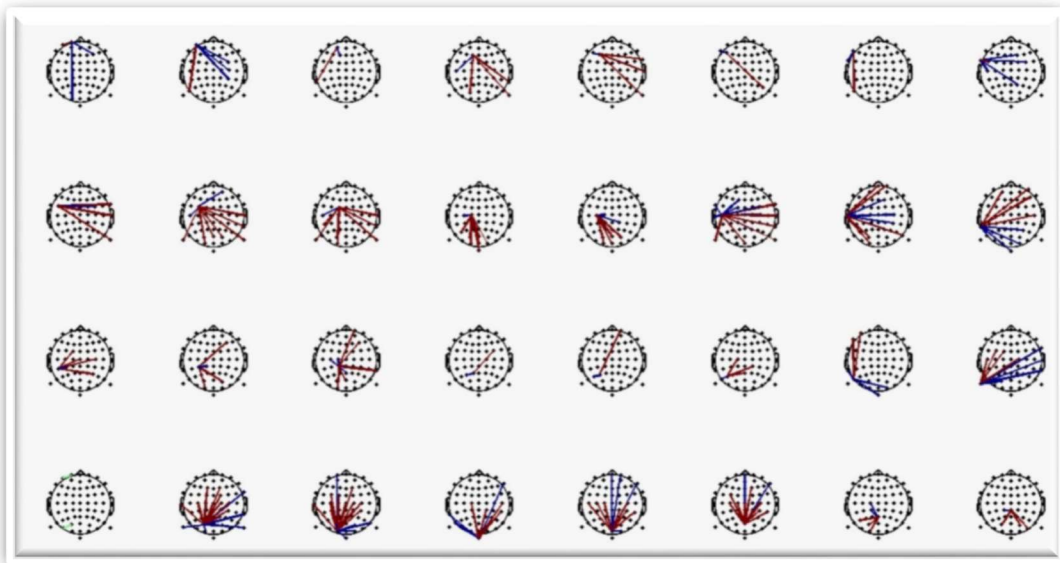


Fig 3.15: Electrode based topography of 1-32 electrodes in delta band

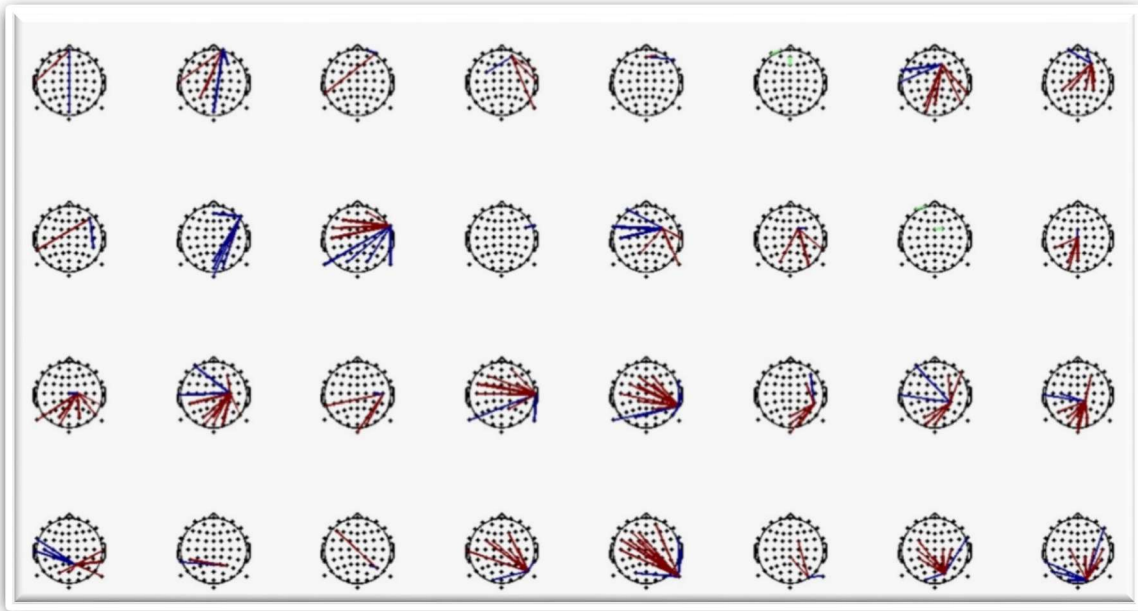


Fig 3.16: Electrode based topography of 33-64 electrodes in delta band

3.3.3.1. Observation:

The red and blue lines correspond to significant connections across all 64 electrodes during sleep stage 1 and sleep stage 2 respectively.

- The less number of red and blue lines shows accordance with our results from our previous plots. Connections are significantly reduced when subjects go to very deep sleep.
- During sleep stage 1 we see a few significant connections coming from right temporal and parietal-occipital region but noticeably none from frontal region.

3.3.3.2. Speculation:

- Absolutely no connections in frontal region accords with our previous study to say anterior default mode network disappears in sleeping stages.

3.3.4. Electrodes showing maximum connections in Sleep-stage1 & Sleep-stage 2

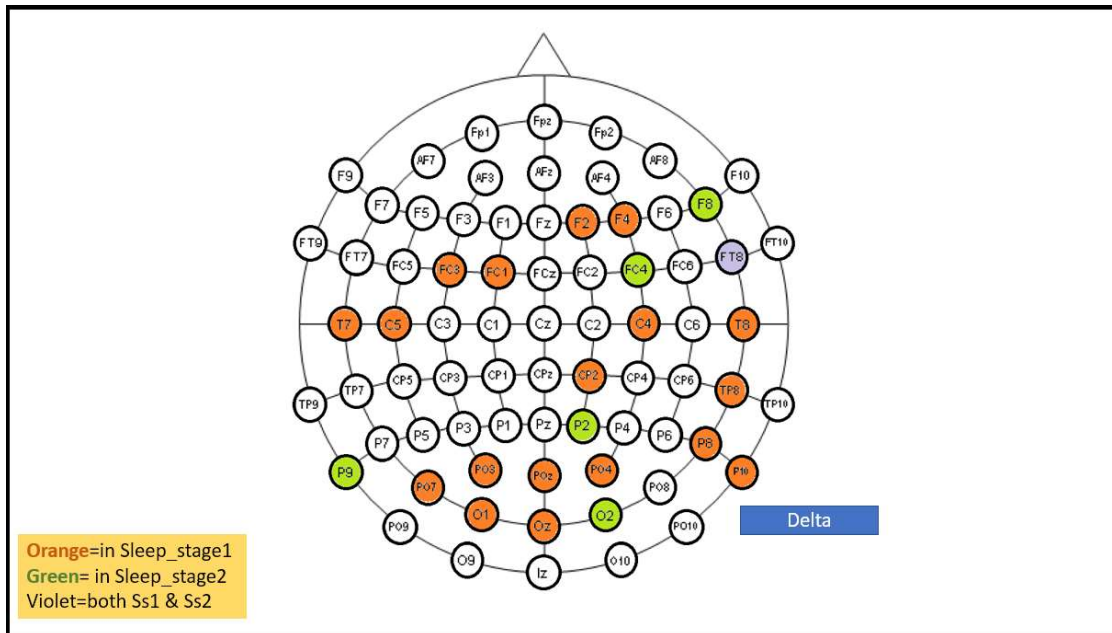


Fig 3.17: Electrodes showing max connections in Sleep-stage1 & Sleep-stage 2

3.3.4.1. Observation:

This is a 2d plot of the entire head to show which electrodes contribute most to significant changes during Sleep-stage 1 and sleep stage 2 across different bands.

Orange and green colored electrodes show major connectivity change during Sleep-stage 1 and sleep stage 2 respectively. And electrodes marked violet show significant changes in both the states.

- None of the electrodes contributing to significant changes in any of the two sleep stages arises from anterior default mode region.
- More number of orange color marked electrodes are prevalent compared to green color marked ones to conclude significantly less brain activities going on during sleep stage 2.

3.4. Alpha band: Gradual change in Default mode network across vigilance states

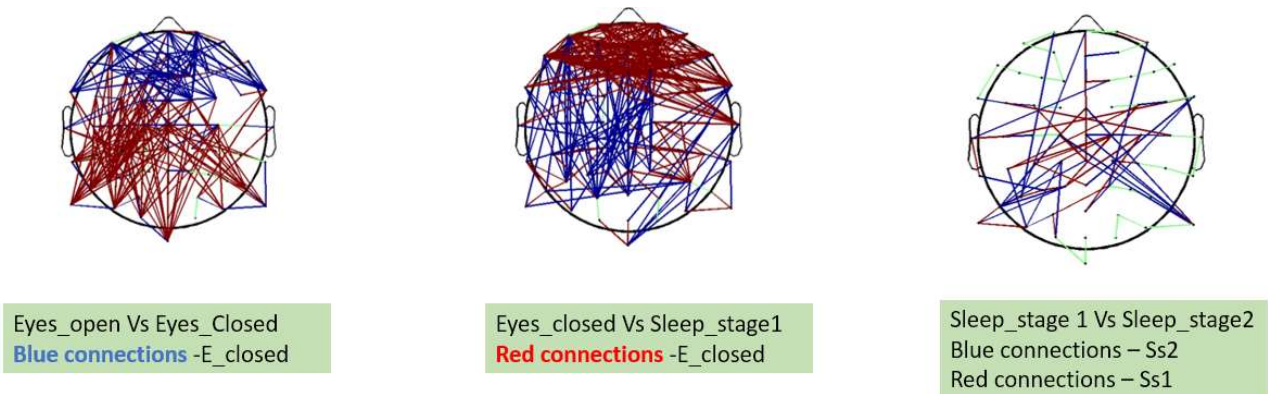


Fig 3.18: Gradual change in DMN across different vigilance states in alpha band

3.4.1 Definition: “The default mode network is an interconnected group of brain regions that seem to show lower levels of activity when we are engaged in a particular task like paying attention, but higher levels of activity when we are awake and not involved in any specific mental exercise. It is during these times that we might be daydreaming, recalling memories, envisioning the future, monitoring the environment, thinking about the intentions of others, and so on--all things that we often do when we find ourselves just "thinking" without any explicit goal of thinking in mind.” [18]

3.4.2. Origin of DMN study: “Although the idea that the brain is constantly active (even when we aren't engaged in a distinct mental activity) was clearly expressed by Hans Berger in the 1930s, it wasn't until the 1970s that brain researcher David Ingvar began to accumulate data showing that cerebral blood flow (a general measurement of brain activity) during resting states varied according to specific patterns; for example, he observed high levels of activity in the frontal lobes of participants at rest. In the early 2000s, Raichle, Gusnard, and colleagues published a series of articles that attempted to more specifically define the areas of the brain that were most active during these rest states. It was in one of these publications that they used the term *default mode* to refer to this resting activity, phraseology which led to the brain areas that exhibited default mode activity being considered part of the *default mode network*.” [18]

3.4.3. Anterior default mode network:

- ✓ **Formation (blue region)** when moving from eyes open to eyes closed state, when the subject is somewhat conscious.
- ✓ Getting even **denser (red region)** during eyes closed state, just before moving over to sleep stages. The reason being eyes closed stage is comparatively more idle state than eyes open.
- ✓ Complete **disappearance** in sleeping stages due to absolute loss in consciousness.

3.5. Glance of Brain network connectivity across in different human vigilance states across four bands

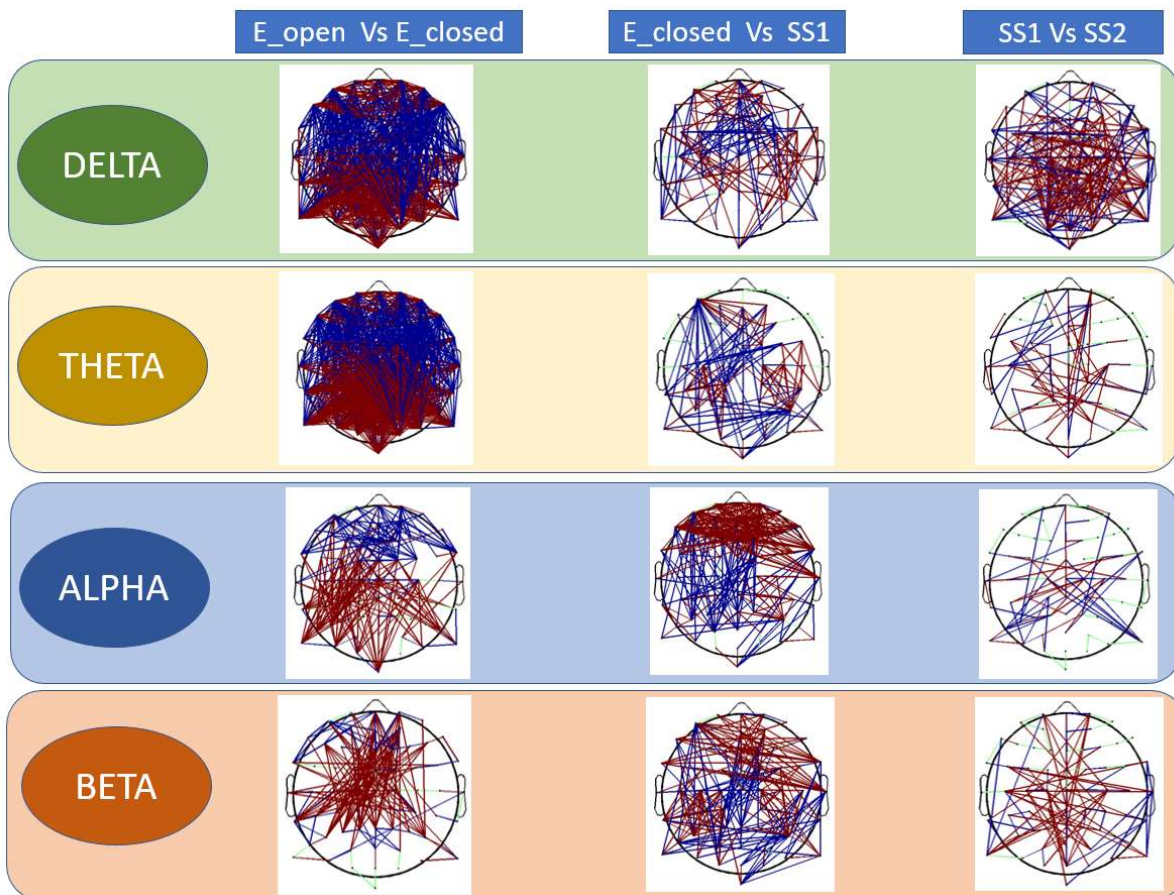


Fig 3.19: Brain network connectivity in different vigilance states across delta, theta and beta bands

CHAPTER 4

DISCUSSION & CONCLUSION

4. Discussion and Conclusion:

In summary, my thesis study showed that brain network connectivity changes significantly when a person gradually moves from a resting eyes-open state to sleep stage 2, as measured with EEG. Furthermore, the study has shown that EEG topo plot analysis can be adapted to investigate various connection strengths across different regions of the human brain.

Specifically, the 64-channel EEG data was preprocessed using brainstorm software where it underwent various filtering, independent component analysis and separation into different bands for performing correlation. The correlation values were then transformed into z-values to make the data normalized before calculating the T-values. Since we are only interested in finding the significant changes in brain connectivity we performed a 2 sample 2 tail t-test to threshold all the insignificant values. These significant T-values were then plotted using colormap plot in MATLAB with positive and negative significant T-values with their electrode locations undisturbed so that by drawing x and y axes to any point in the colormap we know across which pair of electrodes the change has taken place. The topo plot function from EEGLAB software has been modified to plot the connection strengths among different pair of electrodes using significant T-values. With the help of topo plots we get a clear 2d plot of significant connections on the brain which helps us better view and analyze the major regions of connection strengths and how the shifts of connectivity is taking place with change of vigilance states.

During transition from eyes open to eyes closed stage, we see major connectivity alteration in delta and theta bands whereas connections in alpha band were more localized with power stuck at the occipital part due to discontinuity of visual information from front to back when eyes closed. The increase in connections in theta band may be explained from preexisting research studies which proves theta band forms a loop with alpha through which information “reverberates” or

circulates. Also, the default mode network in the anterior prefrontal region has been seen to form in eyes open stage which gets more prominent during eyes closed before disappearing in sleeping stages and one of the important findings from my study reveals that this changes in the anterior default mode network occurs in alpha band. Activation in the motor sensory region decreases when eyes closed since visual information from eyes cannot reach there via occipital part during eyes closed. Delta showing major activation in the sleep stage where there is a significant decrease in connections from stage 1 to stage 2 due to absolute losing of consciousness resulting in lesser brain activity. The connectivity patterns from these vigilance states can be used as a biomarker to have an improved understanding of physio behavioral reasons leading to such change in connectivity patterns which may be helpful in dealing with various sleep disorders. By knowing the healthy connectivity patterns from healthy people and using them as biomarker, noninvasive neurostimulation methods such as transcranial magnetic stimulation (TMS or TES) can be applied on patients with sleep disorders to stimulate necessary regions in their brain to bring back the brain activation to right areas. Notably brain stimulation has been proven to have potentials to treat some disorders such as epilepsy.

4.1. Limitation and Future scope of work:

Since we used correlation method which is a linear measure of connectivity, it did not give us directionality of information flow and we could not infer any distinctions between direct or indirect relations. But correlation is a standard method to identify if there is at all any change in significant connections in human vigilance states, we look forward to addressing the non-linearity and directionality issue in our next study using phase lock synchronization or coherency method as a measure of connectivity.

CHAPTER 5

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6. MATLAB Code:

```
clear all
close all
clc
load('Loc.mat');
load ALPHA1;

% Thresholding T-values
T=ALPHA1;
for i=1:size(T,1)
    for j=1:size(T,2)
        if T(i,j)>-1.7 && T(i,j)<1.7
            T(i,j)=0;
        end
    end
end

figure()
imagesc(T)
colormap winter
colorbar

% Channel Strength

m=size(T,1);
n=size(T,2);
new_array=T;

for i=1:m
    for j=1:n
        if T(i,j)>1.7
            new_array(i,j)=1;
        else if T(i,j)<-1.7
            new_array(i,j)=-1;
        end
    end
end

end

T1.connectStrength=reshape(new_array,64*64,1);
```

channel pair

```
k=1;
for i=1:64:4096

    T1.channelPair(i:i+63,1)=k;
    T1.channelPair(i:i+63,2)=1:n;

    k=k+1;
end
```

Electrode based topography

```
figure()
for ii=1:32
    T2.chanPairs=T1.channelPair(ii*64-63:ii*64,:);
    T2.connectStrength=T1.connectStrength(ii*64-63:ii*64);
    temp=T2.connectStrength;
    temp(find(temp==0))=NaN;
    [T3.chanPairs(:, 1) T3.chanPairs(:, 2)] = ind2sub(size(temp), find(~isnan(temp)));
    xx=T3.chanPairs(:,1);
    T3.chanPairs(:,1)=ii;
    T3.chanPairs(:,2)=xx;
    posi=find(~isnan(temp));
    T3.connectStrength = temp(posi);
    if isempty(T3.connectStrength)
        T3.connectStrength(1)=0;
        yy=[1,2];
        T3.chanPairs=yy;
    end
    if max(T3.connectStrength)==min(T3.connectStrength)
        T3.connectStrength(length(T3.connectStrength)+1)=-T3.connectStrength(1);
    T3.chanPairs(length(T3.connectStrength),2)=ii+1;
    T3.chanPairs(length(T3.connectStrength),1)=ii;
    end
    subplot(4,8,ii)
    topoplot_connect(T3, Loc);
    set(gca,'Clim',[-1 1]);
    clear posi T2 T3 xx temp;
```

```

end

figure()
for ii=33:64
T2.chanPairs=T1.channelPair(ii*64-63:ii*64,:);
T2.connectStrength=T1.connectStrength(ii*64-63:ii*64);
temp=T2.connectStrength;
temp(find(temp==0))=NaN;
[T3.chanPairs(:, 1) T3.chanPairs(:, 2)] = ind2sub(size(temp), find(~isnan(temp)));
xx=T3.chanPairs(:,1);
T3.chanPairs(:,1)=ii;
T3.chanPairs(:,2)=xx;
posi=find(~isnan(temp));
T3.connectStrength = temp(posi);
if isempty(T3.connectStrength)
    T3.connectStrength(1)=0;
    yy=[1,2];
    T3.chanPairs=yy;
end
if max(T3.connectStrength)==min(T3.connectStrength)
    T3.connectStrength(length(T3.connectStrength)+1)=-T3.connectStrength(1);
T3.chanPairs(length(T3.connectStrength),2)=ii-1;
T3.chanPairs(length(T3.connectStrength),1)=ii;
end

subplot(4,8,ii-32)
topoplot_connect(T3, Loc);
set(gca,'Clim',[-1 1]);
clear posi T2 T3 temp xx;

end

```