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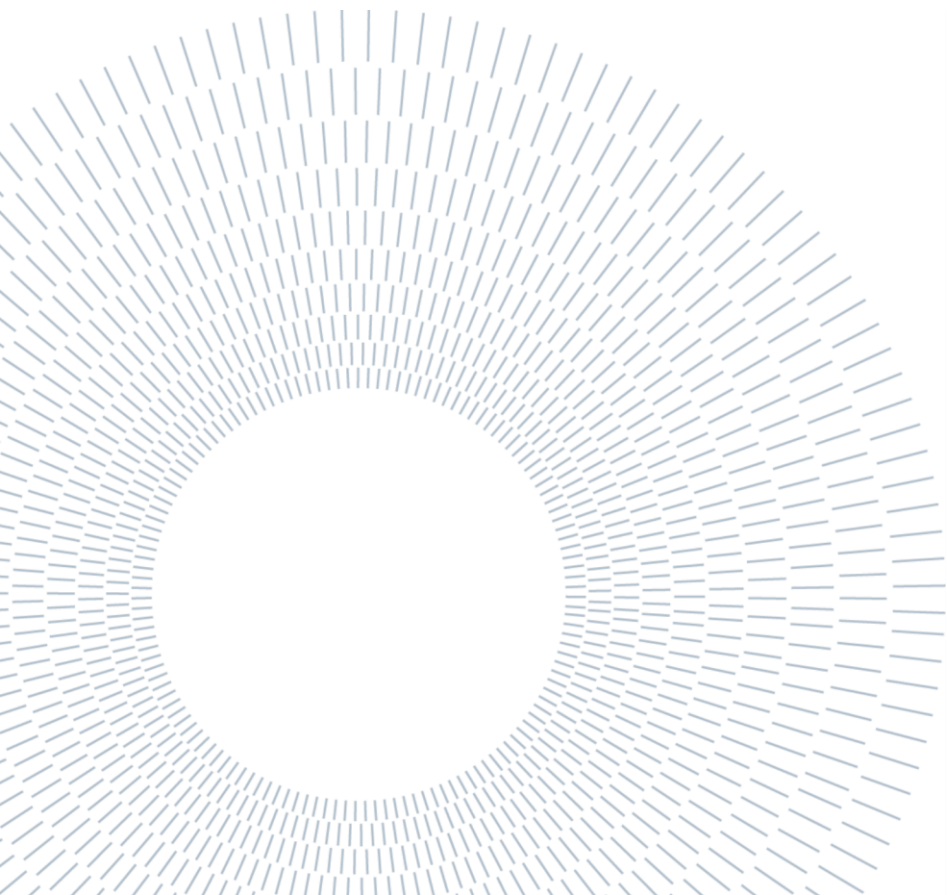
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Analysis of EEG signals for brain connectivity evaluation under emotional stimuli.

BACHELOR'S THESIS IN
BIOMEDICAL ENGINEERING

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Abstract

The following project aims to analyze electroencephalogram (EEG) signals from 31 subjects to evaluate emotions by assessing brain connectivity. Emotions play a crucial role in human experiences as they influence behavior, cognition, and general well-being. Accurate assessment and understanding of emotions are essential in various fields, such as psychology, neuroscience, and human-computer interaction. During this project we study emotions based on the framework that defines emotional states as combinations of two independent dimensions: valence and arousal and attempt to define a model that detects different emotional states using EEG signals.

During the study, subjects are exposed to visual stimuli designed to elicit different emotional states and their EEG signals are recorded and preprocessed. Then, the weighted phase lag index (wPLI) is used to calculate connectivity matrices for each subject, which serve as the basis for subsequent analysis using graph theory measures. By calculating measures such as cluster coefficient, eigenvector centrality and node strength and, after performing an ANOVA statistical analysis, the existence or not of a significant relationship between different emotional valences and EEG signals will be determined to verify whether it is possible to accurately discriminate different emotional states.

This project contributes to the growing field of emotion assessment through EEG analysis using connectivity analysis measures and graph theory, highlighting the complexity of emotion representation in brain connectivity and the need for further exploration using alternative methodologies and measures.

Key-words: Emotions, brain connectivity, valence, graph theory, EEG analysis.

Resumen

El siguiente proyecto tiene como objetivo analizar las señales del electroencefalograma (EEG) de 31 sujetos para evaluar las emociones mediante la valoración de la conectividad cerebral. Las emociones desempeñan un papel crucial en las experiencias humanas, ya que influyen en el comportamiento, la cognición y el bienestar general. Una evaluación y comprensión precisas de las emociones son esenciales en diversos campos, como la psicología, la neurociencia y la interacción persona-ordenador. Durante este proyecto estudiamos las emociones basándonos en el marco que define los estados emocionales como combinaciones de dos dimensiones independientes: valencia y arousal, e intentamos definir un modelo que detecte diferentes estados emocionales utilizando señales de EEG.

Durante el estudio se expone a los sujetos a estímulos visuales diseñados para provocar diferentes estados emocionales y se registran y preprocesan sus señales EEG. Después, se utiliza el índice de desfase ponderado (wPLI) para calcular las matrices de conectividad de cada sujeto, que sirven de base para el análisis posterior con medidas de teoría de grafos. Calculando medidas como el coeficiente de clústeres, la centralidad de los vectores propios y la fuerza de los nodos y, tras realizar un análisis estadístico ANOVA, se determinará la existencia o no de una relación significativa entre las diferentes valencias emocionales y las señales EEG, para verificar si es posible discriminar con precisión los diferentes estados emocionales.

Este proyecto contribuye al creciente campo de la evaluación de emociones a través del análisis EEG empleando medidas de análisis de conectividad y teoría de grafos, poniendo de manifiesto la complejidad de la representación de la emoción en la conectividad cerebral y la necesidad de una mayor exploración utilizando metodologías y medidas alternativas.

Palabras clave: Emociones, conectividad cerebral, valencia, teoría de grafos, análisis de EEG.

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Introduction

In recent years, there has been a growing interest in understanding the functioning of the brain, particularly in relation to emotional processing. Emotions play a vital role in our daily lives, shaping our experiences and how we deal with everyday issues. Therefore the ability to comprehend the underlying neural mechanisms associated with emotional responses holds immense potential for various fields, including psychology, neuroscience, marketing and mental health.

Methods for studying this brain functioning include electroencephalogram (EEG), functional magnetic resonances (fMRI), positron emission tomography (PET) or transcranial magnetic stimulation (TMS), among others. The electroencephalogram (EEG) has emerged as a powerful tool for investigating brain activity and connectivity in response to emotional stimulation. EEG allows for the non-invasive measurement of electrical activity in the brain, offering a high temporal resolution that captures rapid changes in neural dynamics. By examining the brain's electrical signals, researchers can gain valuable insights into the patterns of connectivity and synchronization among different brain regions during emotional processing.

This Bachelor's thesis aims to explore the application of EEG analysis techniques to assess brain connectivity in response to emotional stimulation. The study seeks to address key research questions such as: What kind of connectivity patterns do different emotions trigger? How do these connectivity patterns vary across different emotional states?

To achieve these objectives, the thesis will employ advanced EEG signal processing techniques, using MATLAB. These methods will enable the extraction of relevant features and the quantification of functional connectivity among brain regions. During the study different emotional stimuli will be elicited using images from the IAPS database.

The findings of this research will contribute to the actual knowledge on the neurophysiological underpinnings of emotional processing. By elucidating the brain connectivity patterns associated with different emotional states, this study may provide valuable insights into the mechanisms underlying emotional disorders, such as anxiety and depression. Furthermore, the results may have implications for the

development of personalized therapeutic interventions targeting specific brain networks involved in emotional regulation.

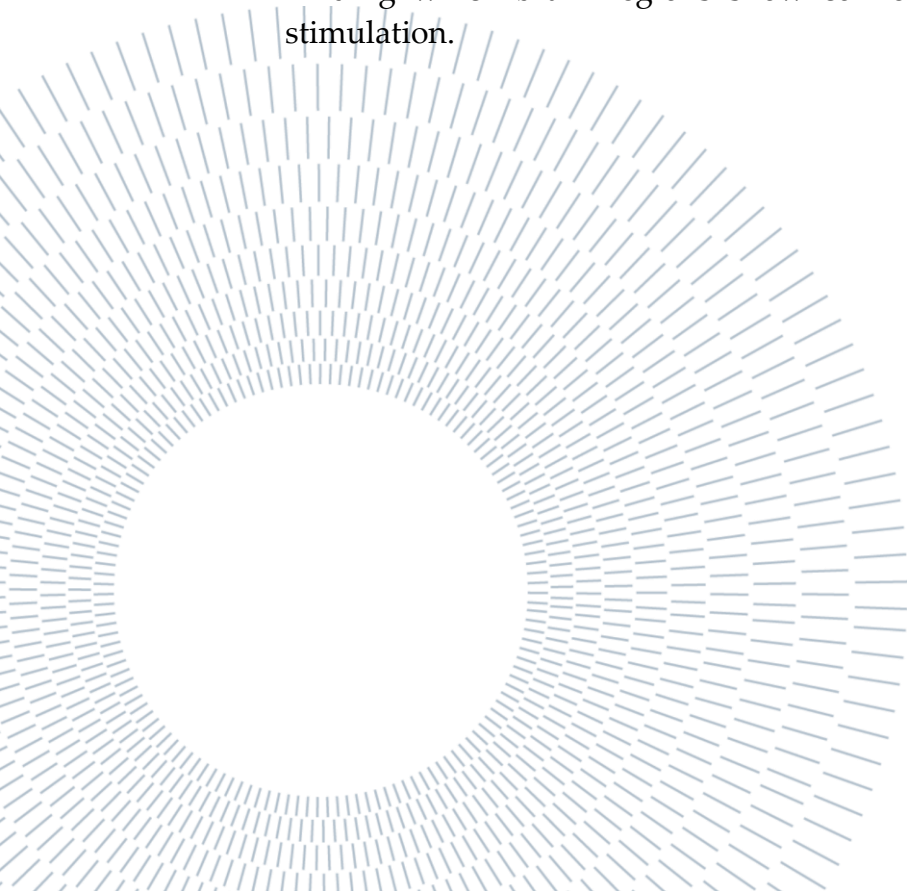
Overall, this Bachelor's thesis aims to utilize EEG analysis to deepen our understanding of brain connectivity in response to emotional stimulation and to develop a model for emotion recognition. By investigating the dynamics of neural networks during emotional processing, we strive to shed light on the complex interplay between emotions and the brain, potentially opening new avenues for improving mental health assessment and treatment strategies.

1. Research aims and objectives.

Emotions have a significant impact on physical and mental health and managing them can improve our life quality significantly. Traditional self-assessments are time-consuming and ineffective because they need voluntary input from the subjects and can be biased by the person filling them. By extracting data from a physiological signal such the EEG, we can monitor the emotional state of the subject in a continuous and more objective way.

This thesis aims to assess connectivity changes elicited by different emotions by analyzing EEG signals from different subjects. The objectives are the following ones:

- Employing effective stimulation protocols for EEG data collection to be used in the models.
- Processing the signals in an optimal way in order to analyze them correctly.
- Selecting the proper features and connectivity analysis methods to develop models for the prediction of valence and arousal responses.
- Finding relations between connectivity patterns and the emotions that elicit them.
- Finding which brain regions show connectivity changes during emotional stimulation.



2. State of the art

2.1. Brain anatomy and functioning.

The brain is the organ that serves as the center of the nervous system. It is the most complex organ of the human body. It controls the functions of the body and allows us to interpret information from the outside world. Emotions, intelligence, memory, creativity... are all governed by the brain.

The brain is composed of cerebellum, cerebrum, and brainstem. The cerebellum is a structure located at the posterior part of the brain, beneath the cerebral hemispheres and above the brainstem. It has been historically associated primarily with motor coordination and balance, but research has revealed that the cerebellum also plays a critical role in various cognitive and emotional processes.

The cerebrum consists of the left and right hemispheres and is the largest part of the brain. They are connected by five commissures, the largest one being a bundle of fibers called *corpus callosum*. In general, the right hemisphere controls functions such as creativity, artistic and musical skills, or spatial ability; while the left one controls speech, comprehension, arithmetic and writing. Each hemisphere is divided into four main lobes: frontal, parietal, occipital and temporal. They all have distinct anatomical features and control specific functions (Kuzniecky & Graeme D. Jackson, 2004).

The frontal lobe is located at the front of the brain, behind the forehead. It is involved in various cognitive functions, including decision-making, reasoning, voluntary movement and problem-solving. It also houses the primary motor cortex, which controls voluntary movements of the body (Augustine, 2017).

The parietal lobe is located between the frontal and occipital lobes, above the temporal one. Its role is to process sensory information from the body, including touch, temperature, pain, and proprioception. It also processes information related to spatial perception, attention, and sensory information – motor function integration.

The temporal lobe is located on the sides of the brain, just above the ears. It is related to auditory processing, memory formation, language comprehension, and some aspects of visual perception. Within this lobe we can find the hippocampus. This is a small structure that plays a crucial role in memory formation, spatial navigation, and certain aspects of emotion, like emotional processing by connecting sensory information with emotional responses (Andersen, 2007).

Finally, the occipital lobe is situated at the back of the brain and is responsible for visual processing. It contains most of the visual primary cortex.

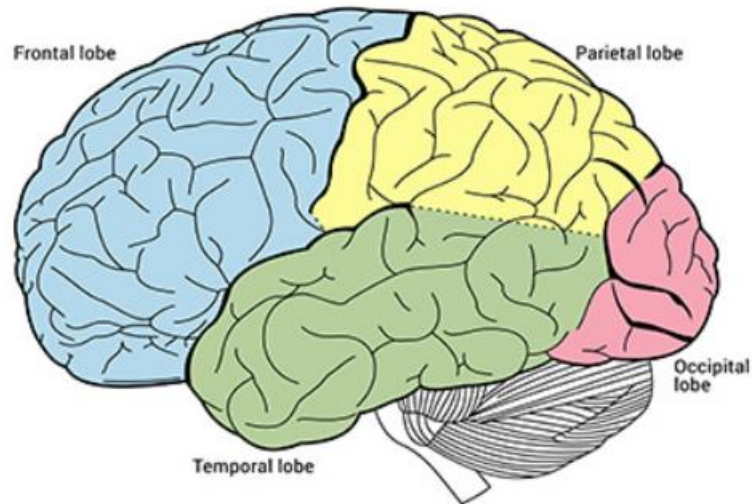


Figure 1. Lobes of the brain. Obtained from <https://qbi.uq.edu.au/brain/brain-anatomy/lobes>

2.2. Brain connectivity

2.2.1. What is brain connectivity?

The term connectivity refers to the communication and interactions between different regions of the brain. In other words, it studies the different pathways through which information is transmitted and processed inside the brain. There are two main types of connectivity: the functional connectivity and the structural connectivity.

The structural connectivity refers to physical connections between different brain regions by neural fibers, known as white matter tracts. It gives us insights on neural architecture. In this case, information transmission is influenced by the condition of a certain pathway of neurons that connect one region with another. Therefore, individual differences in white matter structure could explain variations in behavioral performance and brain disorders related to anatomical connections. This also means that changes in white matter tracts as time goes by can lead to changes in performance of activities, recovery or learning. It is usually studied using diffusion-weighted magnetic resonance imaging, a technique that measures restrictions to local water diffusion (Johansen-Berg et al., 2010).

On the other hand, functional connectivity refers to the statistical dependencies or correlations in the activity patterns of different brain regions. In other words, it can be defined as the temporal correlation between spatially remote neurophysiological events. If there is a statistical relationship between the measures of activities recorded

for two regions, there is functional connectivity. This type of connectivity can be measured using techniques such as functional magnetic resonance imaging or electroencephalography (EEG).

2.2.2. Methods for studying functional connectivity.

There are several methods that can be used to study functional connectivity. In this section we will review some of the main ones.

- **Correlation:** For two different signals A and B, the correlation at each frequency (f) is defined as:

$$r(f) = \frac{C_{AB}(f)}{\sqrt{(C_{AA}C_{BB})}}$$

Being C_{AB} the cross-variance between signals A and B; C_{AA} the auto-covariance of signal A; and C_{BB} the auto-covariance of signal B. This index is sensitive to phase and polarity and can range from -1 to 1.

- **Coherence:** For two signals, A and B, the coherence at each frequency is defined as:

$$Coh(f) = \frac{|S_{AB}(f)|^2}{S_{AA}(f)S_{BB}(f)}$$

Being $|S_{AB}(f)|^2$ the cross-spectral density between signals A and B; S_{AA} is the auto-correlation of signal A; and S_{BB} is the autocorrelation of signal B. This index is sensitive to amplitude and phase change, and its value ranges from 0 to 1. A high coherence means that two brain areas are working closely together but at a specific frequency.

- **Phase synchronization index:** Phase synchronization between two nonlinear oscillation systems is defined as:

$$\varphi_{n,m} = |n\varphi_1(t) - m\varphi_2(t)| < \alpha$$

φ_1 and φ_2 are the phases of two oscillation systems and α is a constant. To compute this, we need to obtain the phase of the signal, which can be defined as:

$$\varphi(t) = \tan^{-1} \frac{x_H(t)}{x(t)}$$

Being $x(t)$ the signal and $x_H(t)$ the Hilbert transform of $x(t)$.

Finally, to compute the phase differences between two signals, we can set $m=n=1$. For these two signals with data length L , the phase synchronization index would be defined as:

$$PSI = \left| \frac{1}{L} \sum_{t=0}^L e^{i\Phi(t)} \right|$$

This index is sensitive to phase change and its value ranges from 0 to 1. A value of 1 means that there is a strict phase locking while a value of 0 means that there are uniformly distributed phases (Lee & Hsieh, 2014).

- Another way of measuring brain connectivity is recording Event Related Potentials (ERP) at each electrode and applying a Discrete Wavelet Transform in order to decompose them into different frequency bands. After that, we can measure the Relative Wavelet Energies as:

$$RE_j = \frac{E_j}{E_{tot}}, j = 1, \dots, 5$$

Being E_j the energy of each frequency band (computed by squaring the coefficients' values) and E_{tot} the total energy (computed by summing the energy values of all the bands).

We can, then, calculate the Relative Energy Distribution of a pair of electrode signals:

$$RWE(p|q) = \sum_{j < 0} p_j \ln \left| \frac{p_j}{q_j} \right|$$

Being p and j two electrode signals.

This is a global synchronization metric which demonstrates the similarity between both signals in terms of all frequency bands. Lower values of RWE indicate higher similarity (Lithari et al., 2010).

- Graph theory measures: Metrics used to analyze the characteristics and properties of a network represented as a graph. They can be applied to study the organization and efficiency of brain networks, understood as nodes representing brain regions, and edges representing connections between them. Some of these measures include clustering coefficient, global efficiency, local efficiency, node degree...

2.2.3. The volume conduction problem.

The volume conduction problem is a fundamental issue and must be taken into account when analyzing EEG signals. This takes place when a brain region generates large electrical signals that can be measured by more than one electrode, and when, due to the conductive nature of the brain or the head tissues (skull, skin of the scalp...), the electrical fields spread “laterally”. This causes that the electrical signals recorded by EEG can be a mixture of activity from different brain regions. In other words, when this happens, connectivity between two electrodes could reflect true connectivity between different brain regions or could be due to two electrodes measuring activity from the same source (Cohen, 2015).

This volume conduction phenomena can be detected when the following patterns are present:

- Zero or π phase lag, because volume-conducted activity is recorded at the same instant at multiple electrodes.
- A decrease in connectivity values with increasing interelectrode distance.
- Volume conduction causes spurious connectivity with only positive correlations.
- Positive correlations between connectivity and power in the same frequency band (Cohen, 2015).

On the other hand, some approaches that can be taken in order to minimize this effect are:

- Applying spatial filters before analyzing connectivity.
- Examining only the negative correlations.
- Testing for temporally lagged connectivity.
- Testing for a cross-frequency correlations.
- Testing for statistical or qualitative differences between the connections between brain regions and their respective power levels.
- Test if the phase lag is significantly different from zero or π , because nonzero phase lag cannot be due to volume conduction.
- Using measures that aren't affected by this phenomenon such as weighted phase lag index.
- Computing correlations between two electrodes while using a constant third electrode (Cohen, 2015)

2.3. Emotions: definitions and theories

Providing a comprehensive definition of what is an emotion is difficult. There are two main theories regarding this issue:

- **Theory of basic emotions:** This theory defends that emotions are a limited set of discrete states, each one independent of the others in its behavioral, psychological, and physiological manifestations. Authors like Ekman defend that there are basic emotions universally recognized across cultures and innate to the human being. The main ones are anger, fear, disgust, sadness, and happiness, and each one activates distinct neural pathways. Ekman also defends that each one of these emotions are related to certain facial expressions and facial muscle movements (Ekman, 1992).

There is a problem with understanding emotions as a set of discrete states because emotions are not always accompanied by explicit behaviors. Also, there are behaviors such as laughing that can be elicited by different emotions (for example, when somebody is happy but also when somebody is nervous). Therefore, the distinction between emotions can be very subtle (Pierluigi Reali, 2021)

- **Dimensional theory of emotions:** This model defines emotions as the result of the combination of two independent dimensions: valence and arousal. In this case emotions are not seen as discrete independent states, but as a continuous spectrum. The valence represents the quality of an emotion and ranges from unpleasant to pleasant. Emotions can be positive, such as happiness, or negative, like anger. On the other hand, the arousal represents the level of activation of an emotion. A low arousal emotion would be calm, and a high arousal one would be excitement, for example. (POSNER et al., 2005).

The valence is usually placed on the horizontal axis and arousal on the vertical axis. The following picture represents this system and how the emotions.

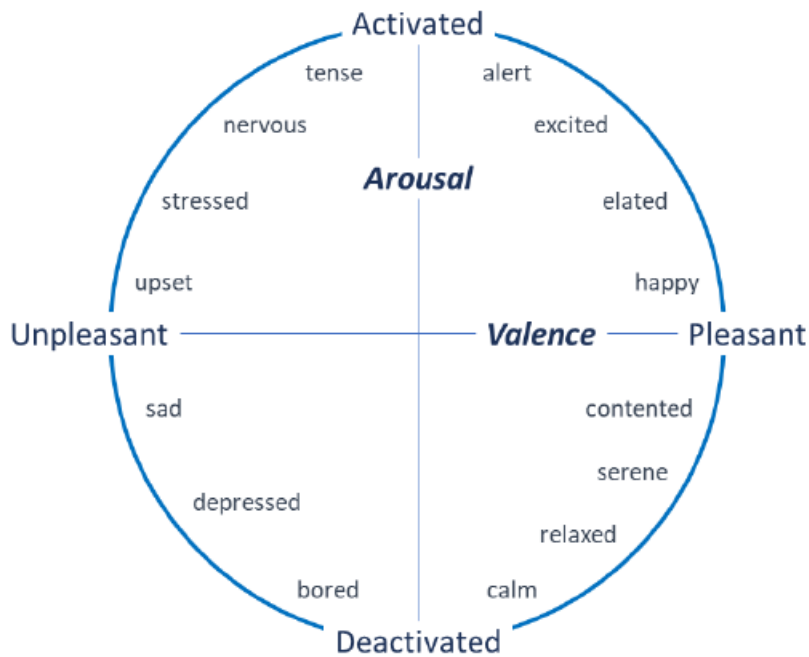


Figure 2. Circumplex model of emotions, with the valence and arousal dimensions (POSNER et al., 2005)

2.4. Previous studies on EEG evaluation to assess functional connectivity in response to emotions and stress.

Most of the studies that intend to find correlations between emotions and EEG signals are focused on EEG characteristics at the single-electrode level. Not many studies have established a connection between brain connectivity and emotional stimulation, using EEG analysis. However, we will review three relevant studies related to this topic.

In their study, Lee and Hsieh use different connectivity indices to recognize emotional states. They analyzed the EEG signals from 40 participants who had watched six emotion-eliciting film clips. The signals were analyzed using correlation, coherence and phase synchronization index for theta, alpha, beta, and gamma bands. An ANOVA was performed with two independent factors: electrode pair and condition (neutral, positive and negative emotion).

In every band, significant differences were found between correlation and coherence patterns and the different emotional states. Also different patterns of the

synchronization index were found in the different bands for different emotional states.

Also, classification performance based on all frequency bands was significantly better than classification performance based on any single frequency band, which indicates the importance of considering all frequency bands in similar studies instead of focusing in just one. The mean accuracy obtained using feature selection was 0.61 for correlation index, 0.62 for coherence and 0.82 for the synchronization index. The better performance of the synchronization index could be explained because coherence index depends on amplitude and phase, and correlation is sensitive to phase and polarity, while phase synchronization is only influenced by the change of phase and therefore reveals clearer information about brain interaction.

Moreover, during the study features from the 19 electrodes were extracted and it was shown that classification performance based on EEG functional connectivity offered better results than that obtained by using single electrodes (Lee & Hsieh, 2014).

In another study, 28 participants viewed emotional pictures and EEG signals were recorded from 19 different electrodes. The signals were analyzed using graph theory metrics such as graph density, clustering coefficient and global efficiency. The Relative Energy distribution was calculated for each pair of electrode signals. After this, different thresholds were used to obtain binary matrices and the graph metrics were computed. The results showed that high arousing pictures produced lower density, Clustering Coefficients and Global Efficiency in the functional connectivity networks. The study concludes that when a human watches high arousing visual stimuli, brain properties at connectivity level seem to be weakened, since Cluster Coefficient and Global Efficiency are reduced (Lithari et al., 2010).

Finally, the study "Graph Analysis on Functional Connectivity Networks during an Emotional Paradigm" showed, using graph metrics, that pleasure modifies the local efficiency of the networks while arousal affected the global efficiency (Lithari, Klados, et al., 2010).

3. Methodology

3.1. Study design.

The people recruited for the study were 22 healthy men and 9 healthy women, who were part of the Bioengineering class of the Politecnico di Milano, aged between 18 and 27 years (being 20.9 the mean age and 1.5 the standard deviation). They all were from the same course because the intention was to increase the sample homogeneity, reducing inter-subject variability of the emotional response. They all were asked to avoid alcohol, coffee, smoke and tea for the 4 hours previous to the study (Pierluigi Reali, 2021).

All of them were shown 90 different images from the IAPS database. The IAPS database is a standardized collection of images used in research related to emotions and affective processing. Each image has been carefully selected and rated based on its emotional content. Therefore, the images shown to each participant had been previously classified by valence and arousal into 9 different groups: high arousal and high valence (HA-HV), high arousal and medium valence (HA-MV), high arousal and low valence (HA-LV), medium arousal and high valence (MA-HV), medium arousal and medium valence (MA-MV), medium arousal and low valence (MA-LV), low arousal and high valence (LA-HV), low arousal and medium valence (LA-MV), and low arousal and low valence (LA-LV). Each of these blocks is supposed to elicit similar emotions (Pierluigi Reali, 2021).

Before this, each participant was asked to close the eyes for 60s and, after that, a 90-second neutral image was shown to help them relax. Also, between each arousal-valence group of pictures, a 30s neutral image was shown. This picture consisted of a wooden surface and its goal was to avoid eliciting any particular emotion during watching. Every person was shown a block of 10 pictures from the same arousal-valence group, each of them lasting 12s (making each arousal-valence block 2 minutes long). For each participant, the different groups of images were shown in an increasing order of arousal in order to maintain concentration. Thus, the LA-HV, LA-MV and LA-LV blocks were shown first; then the medium arousal ones; and finally, the high arousal blocks. However, the order in which images with different valences were displayed within an arousal group changed from one subject to another. For example, the order of the low arousal groups for a participant could be LA-HV, LA-MV, and LA-LV and the order for another participant could be LA-MV, LA-LV, LA-HV. The EEG was collected throughout the entire procedure (Pierluigi Reali, 2021)

An outline of the stimulation sequence followed during the experiment is shown below.

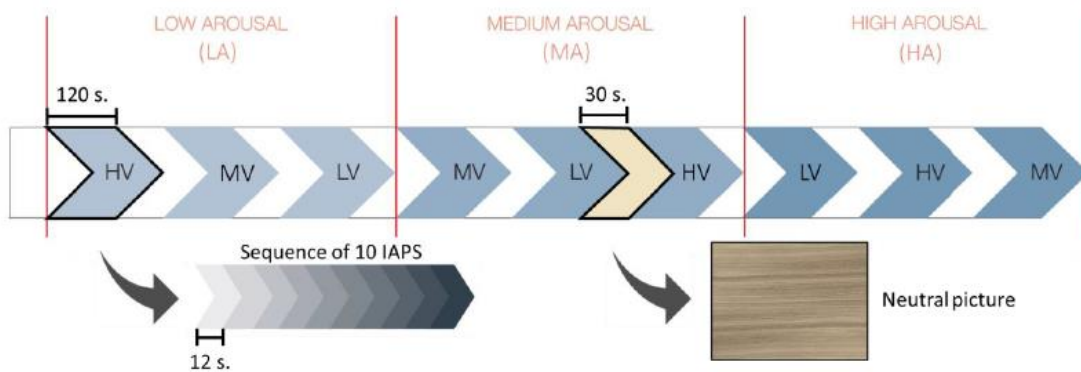


Figure 3. Stimulation sequence of the experimental protocol (Pierluigi Reali, 2021).

3.2. EEG data acquisition and processing.

In this research, emotions experienced by the subjects during the study will be assessed from brain signals collected by an EEG for each subject. Brain signals were recorded from 25 different electrode places according to the 10/10 system. This system is a standardized method used for electrode placement in electroencephalography (EEG) recordings. It refers to a system where electrodes are positioned at specific locations on the scalp based on a set of predefined landmarks. The "10/10" notation indicates that the electrode positions are determined by dividing the head into a grid with 10% and 20% intervals. The electrodes in the 10/10 system are labeled with a combination of letters and numbers. The letters represent the region of the head, and the numbers represent the specific electrode position within that region. The letters "F," "T," "C," "P," and "O" represent frontal, temporal, central, parietal, and occipital regions, respectively. The number "1" denotes an electrode placed at the 10% distance from the nasion, and "2" denotes an electrode placed at the 20% distance. (<https://www.sciencedirect.com/science/article/pii/S1053811906009724>)

The electrodes used as recording channels were: Fpz, Fp1, Fp2, AF3, AF4, AF7, AF8, Fz, F1, F2, F3, F4, F5, F6, F7, F8, Cz, C3, C4, T3, T4, T5, T6, O1, and O2. the reference electrode was placed at and the sampling rate was 256 Hz.

The positions in which the electrodes were placed are shown below:

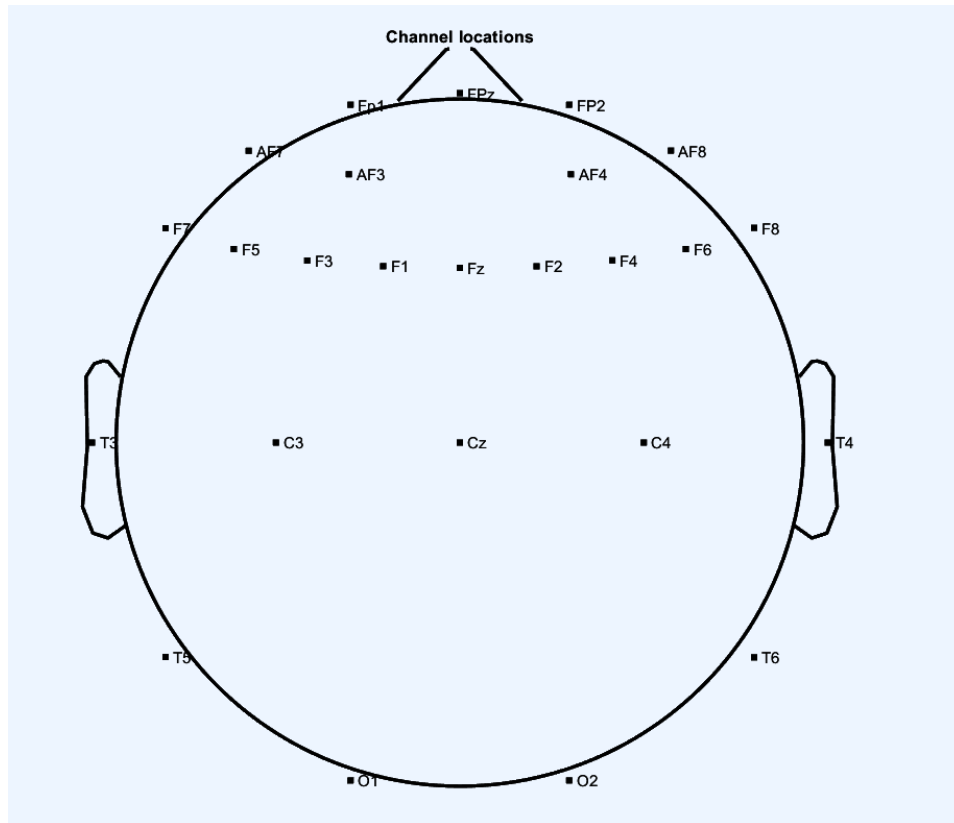
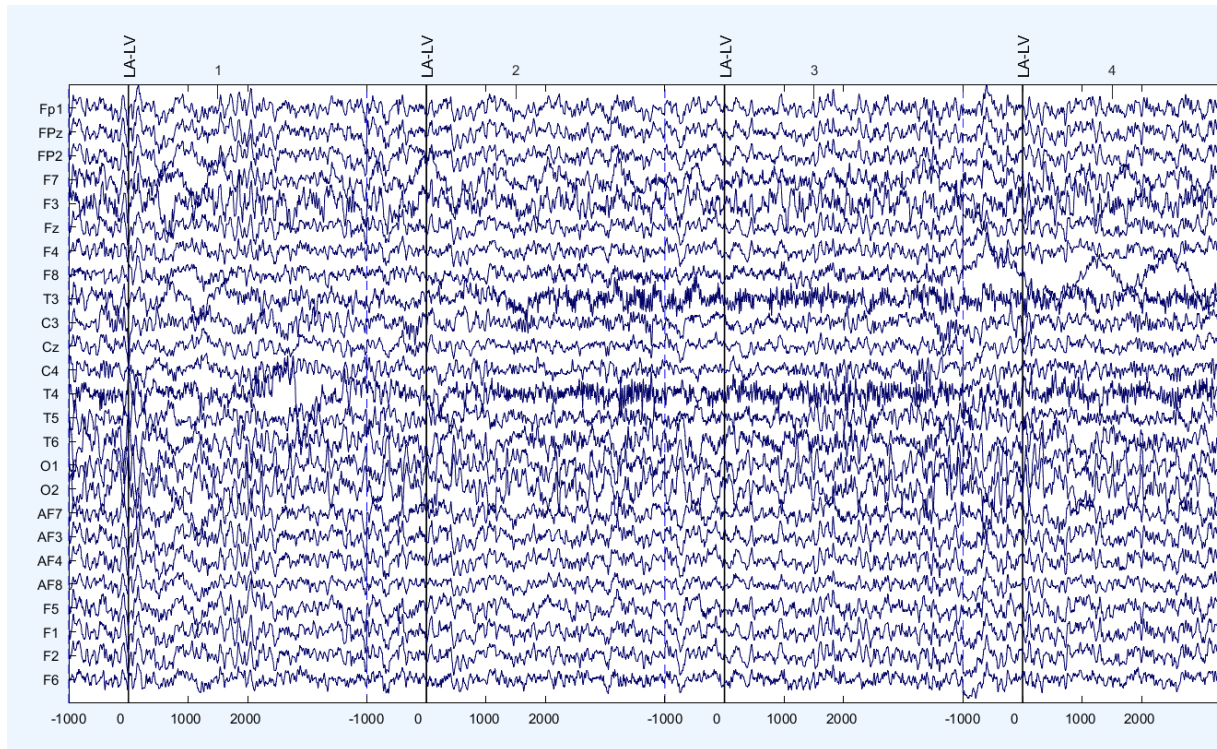


Figure 4. Position of the recording channels obtained with EEGLAB. Source: MATLAB

EEG signals were pre-processed using EEGLAB toolbox and custom scripts optimized for the study aim. First, data were band-pass filtered between 1 and 45 Hz with a Finite Impulse Response (FIR), zero-phase filter and bad channels were visually selected and removed. Signals were cut into epochs from -1 to +4 seconds with respect to the stimulus presentation. The extended Infomax independent component analysis was applied to the concatenated epochs and with the support of the IClab plugin the sources of artifacts were identified and removed. The previously rejected bad channels were interpolated, and signals were re-referenced to the infinite reference using the Reference Electrode Standardization Technique (REST plugin). Finally, epochs with residual artefacts were visually checked and rejected.

Each epoch corresponds to an image from a certain valence and arousal work. The recording of each epoch started 1s before the stimuli and ended 4 seconds after it. The sampling rate was 256 Hz so the frames per epoch were 1280. An example of how each epoch is recorded for each channel is provided below:



F

Figure 5. Representation of how the different epochs are recorded for each channel obtained with EEGLAB. Source: MATLAB

3.3. Methods used for studying brain connectivity.

3.3.1. Weighted Phase Lag Index.

First, we use MATLAB and the package EEGLAB to load and analyze the different datasets. For each kind of event, a set of data corresponding to the rest (1s before the stimuli) and a set of data after the stimuli are extracted. The data set corresponding to the stimulus only takes into account the information between 500 and 1500ms after the stimulus.

After that, we used the script wPLI_Hermes from the Hermes Toolbox. It is used to calculate the weighted Phase Lag Index (wPLI). The wPLI is a measure of functional connectivity used in EEG signal analysis and represents the asynchrony and strength of synchronization between different brain regions. This index is useful for investigating functional connectivity in the brain and can provide information about the dynamic interactions between different brain regions. It is commonly used in neuroscience studies to analyze the synchronization between brain areas in different experimental conditions or cognitive states, allowing researchers to examine the network dynamics and information flow within the brain.

The wPLI is an extension of the PLI. The PLI is calculated from the instantaneous phases of two time-series (in our case, it is the signal of two different electrodes), and can be expressed as:

$$PLI = | \langle \text{sign}[\sin(\Delta\varphi(tk))] \rangle | \quad (\text{Hardmeier et al., 2014})$$

Being $\Delta\varphi$ the phase difference at time-point k between two time series, determined for all time points per epoch, $\langle \rangle$ denotes the mean value and $||$ indicates absolute value (Hardmeier et al., 2014).

The PLI index characteristics lead to sensitivity issues with the volume-conduction problem and noise. Small perturbations turn phase lags into leads and vice versa, therefore reducing the capacity to detect changes in phase-lag synchronization. The WPLI addresses this problem by weighting the contribution of phase leads and lags based on the magnitude of the imaginary component of the cross-spectrum. The WPLI offers reduced sensitivity to noise sources and increased statistical power for detecting changes in phase synchronization compared to the PLI (Vinck et al., 2011)

The wPLI provides a value between 0 and 1, where 0 indicates no phase synchronization and 1 indicates perfect synchrony. Intermediate values reflect the degree of phase synchronization between brain regions.

The *wPLI.m* script applies a Fourier Transformation to obtain frequency components and then calculates the phase of each frequency component for each pair of channels. Using phase differences, it estimates the asynchrony of each pair of channels and weights them. What we get after using it is a weighted connectivity matrix, where each element represents the strength of synchronization between channel pairs as a function of their phase asynchrony.

Once we calculated this index for the beta, theta, and alpha frequency bands, we did different visual comparisons. First, we compared the indexes calculated during rest and after the stimulus for each band for every subject. The idea is to find out if there really is a difference in connectivity between resting and stimulated states. We plotted a 25x25 matrix that showed the wPLI index for each channel pair, assigning a different color according to the value obtained, ranging from blue for value 0 to yellow for value 1. Therefore, the rows and columns of the matrix denote nodes (the recording channels) and the matrix entries denote links between them.

We then compared the wPLI between the different bands for events associated with different valences. In this way, we were able to analyze whether there were significant differences between connectivity when viewing emotion-eliciting images associated with different valences. For this comparison, we considered only high

arousal events, as they showed more differences between groups of events of different valence. We used the same 25x25 matrix as before.

3.3.2. Right and left hemisphere connectivity and interconnectivity calculation.

Once the connectivity matrix was calculated for each subject, an additional value was calculated. We computed the average connectivity values from the frontal right hemisphere and for the frontal left hemisphere, and the average interconnectivity value.

In order to calculate the average right frontal hemisphere connectivity, the first thing we did was to extract the connectivity values from the connectivity matrix for the connections involving the electrodes placed in the right frontal part of the brain. These electrodes are Fp2, AF4, AF8, F2, F4, F6, and F8. After that, to extract the average connectivity value, we just took the mean of these values.

To calculate the average left frontal hemisphere connectivity value we did the same, but now extracting from the matrix the values corresponding to the left frontal side of the brain. In this case, we use the values from the connections between the electrodes Fp1, AF3, AF7, F1, F3, F5, and F7.

Finally, to compute the frontal interconnectivity, we extracted the connectivity values corresponding to the links between an electrode from the frontal right side and another from the frontal left side. Afterwards, we calculated the mean of these values.

3.3.3. Graph measures.

Another way to study connectivity is using graph theory measures. Graph theory is a branch of mathematics that deals with the study of graphs, which are mathematical structures used to represent relationships or connections between objects. A graph consists of a set of vertices (also called nodes) and a set of edges (also called links or connections) that connect pairs of vertices. In the context of network analysis, graph theory provides a framework for analyzing and understanding the structure and properties of complex networks. It allows researchers to examine the patterns of connections, the flow of information, and the interactions between nodes in a network (Ronald Gould, 2012).

In the context of brain connectivity, graph theory provides a valuable framework for analyzing and understanding the complex network of connections between different regions of the brain. The brain can be represented as a graph, where each region or node represents a specific brain area, and the edges represent the functional

connections between these areas (Friston, 1994). Links between the nodes can be differentiated based on weight and directionality. Binary links denote the presence or absence of connection while weighted links also contain information about the strength of the connection (Rubinov & Sporns, 2010). During the present study weighted links will be used, as they provide a more detailed characterization of the connectivity patterns. Links may also be characterized by the presence or absence of directionality (Rubinov & Sporns, 2010). In this case we will use undirected links.

A graphical example of how these networks are represented is shown below.

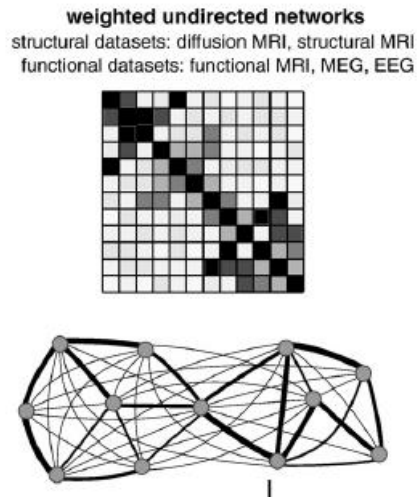


Figure 6. Representation of a weighted undirected network.

There are many different measures used to characterize these networks (centrality measures, integration measures, resilience measures, segregation measures...). In this case we will focus on four of them: node strength, node betweenness, eigenvector centrality and cluster coefficient.

3.3.3.1. *Node strength*

A basic and important measure on these networks is the degree. In a binary network, the degree of an individual node is equal to the number of links connected to that node or, in other words, the number of neighbors that it has. In case of weighted networks, the weighted variant of the degree is also called strength. In this case, node strength is calculated by summing the weights of all the connections that a particular node has with its neighboring nodes and represents the cumulative effect of the node's interactions with others in the network (Rubinov & Sporns, 2010). It can provide insights into the activity level of a node in the network since nodes with higher strength values have stronger and more significant connections with other nodes, indicating their potential importance or centrality in the network structure.

To calculate node strength, we can use the following formula:

$$Strength(i) = \sum A(i,j), \text{ for all } j$$

Being strength(i) the strength of node i, and A(i,j) the weight of the connection between nodes i and j.

In other words, using a connectivity matrix previously calculated, the strength of a certain node could be calculated by summing all the values in the corresponding row (or column) of the connectivity matrix. For example, to calculate the strength of the brain area corresponding to the electrode F5 we will look what row in the connectivity matrix corresponds to this electrode and sum all the values in that row.

3.3.3.2. Clustering Coefficient

Functional segregation of the brain refers to the specialization and division of different brain regions or networks for specific cognitive or functional processes. It is the idea that different regions of the brain are responsible for different functions and work together in a coordinated manner to support various cognitive processes. This specialized processing occurs within densely interconnected groups of brain regions, known as clusters. In other words, clusters refer to groups of brain regions that exhibit stronger and more dense connections among themselves compared to connections with regions outside the cluster. These clusters represent functionally related brain regions that are believed to work together to perform specific cognitive or functional processes (Zemanová et al., 2006).

Regarding this clusters, we can define the clustering coefficient as the fraction of triangles around an individual node. It is equivalent to the fraction of the node's neighbors that are also neighbors of each other and it measures the tendency of the nodes to form tightly connected clusters of groups (Watts and Strogatz, 1998). The mathematical formula for the clustering coefficient is the following:

$$C^w = \frac{1}{n} \sum_{i \in N} \frac{2t_i^w}{k_i(k_i-1)} \quad (\text{Rubinov \& Sporns, 2010})$$

Where N is the set of all nodes in the network, and n is the number of nodes. C_i is the clustering coefficient of node i and k_i is the degree of the node (number of links connected to the node).

3.3.3.3. *Node betweenness centrality.*

Important brain regions often interact with many other regions and facilitate functional integration. Centrality measures are quantitative metrics used in network analysis to assess the importance, influence, or prominence of nodes within a network. These measures help identify the most central or influential nodes in a network based on their connectivity patterns and their role in facilitating communication, information flow, or resource transfer.

Many of these measures are based on the idea that central nodes participate in many short paths within the network (Freeman, 1978). Taking this into account, betweenness centrality quantifies the extent to which a node acts as a bridge or intermediary between other nodes. It captures the number of shortest paths between pairs of nodes that pass through a given node. When a node has a high betweenness centrality it means that it participates in a large number of shortest paths and, usually, that it is a bridging node which facilitates communication and information flow across the network. Therefore, this measure could be helpful to detect nodes that connect different brain regions and facilitate communication between them. It can be calculated as:

$$b_i = \frac{1}{(n-1)(n-2)} \sum_{\substack{h,j \in N \\ h \neq j, h \neq i, i \neq j}} \frac{\rho_{hj}(i)}{\rho_{hj}} \quad (\text{Rubinov \& Sporns, 2010})$$

Being n the number of nodes, and ρ_{hj} the number of shortest paths between h and j , and $\rho_{hj}(i)$ the number of shortest paths between h and j that pass through i .

3.3.3.4. *Eigenvector centrality.*

Eigenvector centrality is another centrality measure. It is a measure of node importance or influence within a network. It is based on the concept that the importance of a node in a network is influenced by the importance of its neighboring nodes (Bloch et al., 2023). In other words, a node is considered more central if it is connected to other central nodes and, therefore, nodes have high eigenvector centrality if they connect to other nodes that have high eigenvector centrality. Nodes with higher eigenvector centrality are considered more influential within the network. Having connections to other highly central nodes, which enhances their influence and allows them to effectively spread information or control the flow of information through the network.

If A is an adjacency matrix of the brain network that we are studying (in our case, the connectivity matrices that we have previously calculate. Here, the eigenvector centrality is defined as the eigenvector associated with the largest eigenvalue λ of the matrix A (Bonacich and Lloyd, 2015). This means that the eigenvector centrality of a

node i is equivalent to the i^{th} element of this vector, and can be computed using the following equation:

$$x_i = \frac{1}{\lambda} \sum A_{ij} \cdot x_j$$

Being x_i the eigenvector centrality of node i , λ the dominant eigenvalue of the adjacency matrix A , A_{ij} the element in row i and column j A , and x_j the eigenvector centrality of node j .

This is an iterative process where the centrality of each node is calculated based on the centrality of its neighbors. This process is repeated until convergence is reached, ensuring that the centrality values stabilize.

3.4. Graphical representation.

Once all these values have been computed, we perform a series of graphical analyses to look for patterns that can relate the values of the measurements taken to emotional states (through the valence of emotions) or to states of rest and arousal. We also seek to analyze if there is any relationship between the cerebral hemisphere and the connectivity or characteristic patterns in one or the other hemisphere, which allow us to identify these emotional states.

First, for each subject we plot their connectivity matrix for different conditions. On the one hand, these matrices are plotted, with colors in each input dependent on the connectivity value, for all frequency bands and in the stimulus state, for emotional states corresponding to different valences (all high arousal). Thus, the aim is to visually compare the matrices of each subject for each state and try to detect patterns that allow us to distinguish the different emotions. On the other hand, another figure shows, for each subject, several matrices corresponding to the connectivity matrices obtained, for each frequency band and each type of stimulus, during the resting and stimulation states. This is intended to assess, in the same way as before, whether there is any visually obvious difference between resting and stimulation.

Next, we took the average connectivity values of the right hemisphere of each subject and plotted them on a raincloud plot. This is used to display the distribution of a continuous variable across different groups or categories. The x-axis shows the different valences of emotional stimuli: HV, MV and LV (all for high arousal states). On the y-axis, for each emotional state, a point representing the connectivity value of this hemisphere for each subject is plotted. In addition, the values obtained during resting states are plotted in red and those obtained after stimulation are plotted in blue. Thus, we have 3 categories on the x-axis, and for each of them, 62 points on the y-axis (31 blue and 31 red). In this graph, apart from the individual points, a density trace is represented. This represents the underlying distribution of the data and is

displayed as a smoothed kernel density plot, showing the probability density of the variable. This plot is repeated for each of the frequency bands. In addition, for each band, a raincloud plot representing the connectivity variation between the stimulation and the resting state is also plotted.

This is then repeated for the left hemisphere and for the connectivity between hemispheres. What we intend to do is to be able to visually relate the average connectivity of the participants with the cerebral hemispheres, with the different valences or with the states of rest and stimulation.

Furthermore, regarding the graph measures we did two graphical studies. First, using the MATLAB function `topoplot`, we plotted a topographic map on a 2D circular view, for each one of these metrics, which showed the spatial distribution of the mean value of the different metrics across the scalp electrodes. To create this, we first need to define the spatial locations of each electrode. This map is color-coded, and the color intensity or contour lines represent the magnitude or distribution of the variable. This allows us to visualize how the measure varies across different scalp locations and may be useful to find spatial patterns associated with different cognitive or experimental conditions. That is why we plotted one for each frequency band, and each emotional valence, and for both rest and stimulation states (we also plotted one map for the difference between the values during stimuli and during rest), aiming to find any relationship among them.

In a similar way as we did with the mean connectivity values, we also made a raincloud plot corresponding to each cerebral hemisphere, for each of these graph measures: clustering coefficient, node total strength, betweenness centrality and eigenvector centrality. To do this, as we did before, we must first calculate the mean value of each measure for the electrodes on the right side and those on the left side.

Finally, we visualized different boxplot graphs in order to better analyze the distributions of the different metrics. First, we created a figure with three different graphics, one for each frequency band, each one containing six boxplots. The boxplots represented: the right-side connectivity, the left-side connectivity, and the interconnectivity during rest, and the same measures during stimuli. This was repeated for each of the three valences: HV, MV and LV.

Then, for each of the graph measures, we also created a figure with three graphics, each one for a frequency band, each containing six boxplots. In this case, each boxplot contained the distribution of a determined measure, for example cluster coefficient, for each emotional valence during rest and stimuli. We did one figure for the left hemisphere and one for the right one, so we could compare them.

3.5. Statistical analysis.

Finally, in order to perform a more robust analysis, an ANOVA statistical study was carried out. A hand-two side repeated measure ANOVA was computed. With this analysis we compare the mean differences between groups that have been split on two within-subject factors. In our case, we used two independent factors: brain side and valence. We want to asses if there are significant effects of each variable on the previously calculated values, and whether if there is a significant interaction effect between the two variables. In other words, with this analysis we will assess the effect of brain hemisphere and emotional valence, and the interaction between them, in the different measures obtained (connectivity values and graph measures). For each metric and for each independent factor (and also for the interaction between them) we calculate the p-value and compare it to a significance level of 5%. Therefore, we will consider a significant interaction if the p-value calculated is lower than 0.05.

3.6. Software tools.

All calculations and graphs mentioned above have been performed using MATLAB. Below are some of the packages used and what they were used for:

- EEGLAB: It is a widely used MATLAB toolbox used for processing and analyzing EEG data. It was used to preprocess the data obtained during the study, after importing and visualizing it.
- Hermes Toolbox: It is a MATLAB package that includes several scripts that allowed us to calculate functional connectivity from the clean EEG signals we had obtained. One example of these functions was the *wPLI_Hermes.m* which applied the weighted Phase Lag Index in order to calculate the connectivity values.
- 2019_03_03_BCT: The Brain Connectivity Toolbox is a MATLAB package used for complex graph analysis of the functional brain connectivity. It contains specific functions to calculate, using the values of the functional connectivity for each electrode pair, network measures such as strength, clustering coefficient or eigenvector centrality.
- To visualize the rain cloud plots mentioned earlier, the function *rm_raincloud_mod.m* was used. It was extracted from the package RainCloudPlots-master which is a toolbox designed for creating this kind of plot.
- Finally, in order to perform the repeated measure ANOVA, we used MATLAB functions such as *ranova.m* or *mulcompare.m*.

4. Results.

4.1. Results of the research

After carrying out the measures described in the previous section, we have obtained the following results.

First, by applying the Wpli, we obtain, for each subject, a figure containing nine connectivity matrices, as follows.

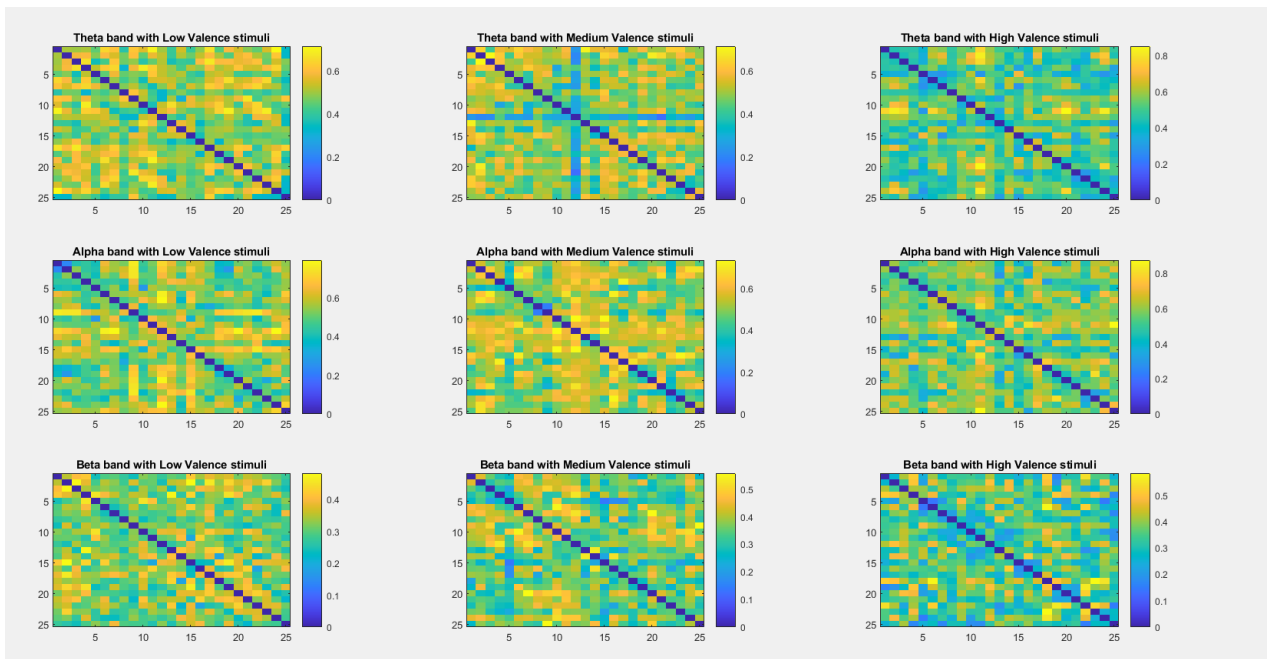


Figure 7. wPLI value comparison among frequency bands for events with different associated valences for one patient. Source: Matlab

In this case we compare the different emotional valences for each subject. As mentioned before, each point represents a functional connectivity value for the given pair of electrodes, ranging from dark blue for values close to 0, to a yellow color for high values (close to 1). A matrix like this has been plotted for all 31 subjects and no relationship or pattern has been found at the visual level.

The equivalence between the number shown in the graphs and the electrode to which it refers is shown in the following table:

Number	Electrode
1	FP1
2	FPz
3	FP2
4	F7
5	F3
6	Fz
7	F4
8	F8
9	T3
10	C3
11	Cz
12	C4
13	T4
14	T5
15	T6
16	O1
17	O2
18	AF7
19	AF3
20	AF4
21	AF8
22	F5
23	F1
24	F2
25	F6

Table 1. Equivalence between electrode name and numbers shown in the figures.

We now show the results obtained, for a given subject, by calculating six connectivity matrices comparing the states of rest and stimuli for the different frequency bands.

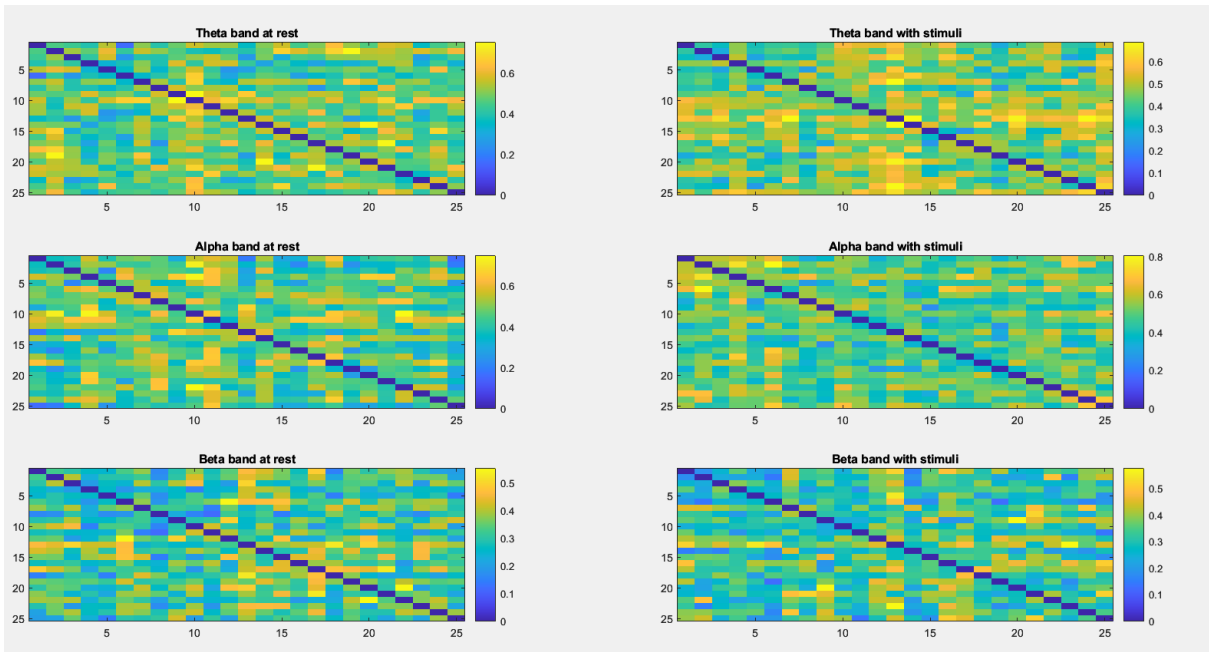


Figure 8. wPLI value comparison among frequency bands between rest and after stimulation states for one patient. Source: Matlab

The equivalence between the electrodes and the numbers is the same as shown above. As before, it has not been possible to visually detect common patterns among subjects that allow us to differentiate between rest and stimuli states.

Next, we can see the topographic maps plotted. As we have said, these maps are a visualization technique to study the distribution of one of the measures taken across the scalp. For example, here we can see what we obtained for the clustering coefficient values across the beta band:

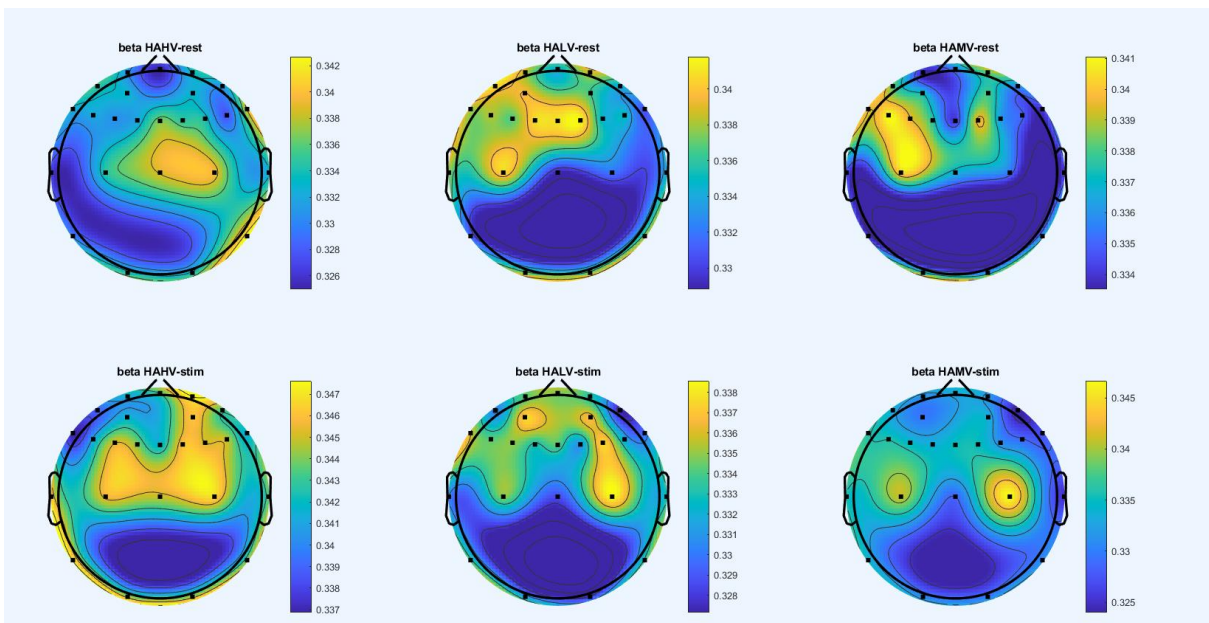


Figure 9. Clustering coefficient values across the scalp representation. Source: Matlab

Although there seem to be differences between the different emotional valences and states of rest and arousal, if we look at the numerical values of each graph, we see that they are almost identical, so these differences are almost nil. In fact, if we establish the color scale for values between 0 and the value of the clustering coefficient, it is clear that the values are practically the same over the entire surface.

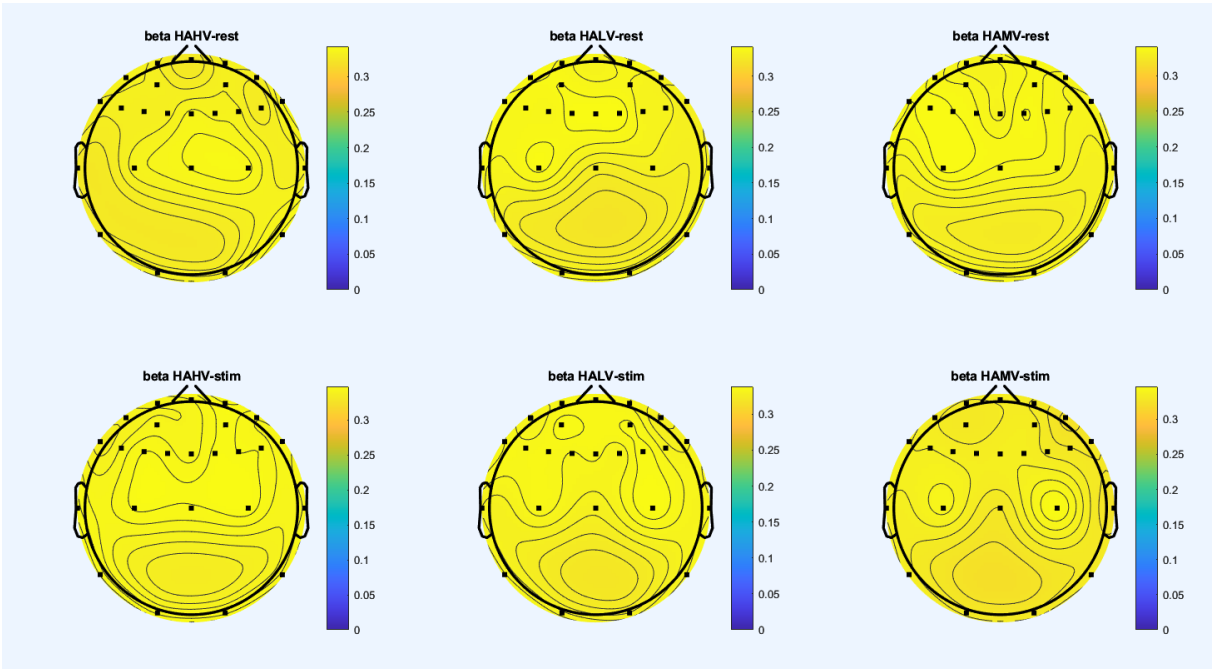


Figure 10. Clustering coefficient values across the scalp with the scale starting from zero. Source: Matlab

We repeated the measures for the other bands and for the other metrics (eigenvector centrality, node strength, connectivity values...) and the results obtained were very similar.

As we have explained in the previous section, we also plotted a raincloud plot for the average connectivity values of each subject in each brain hemisphere. The results are shown below:

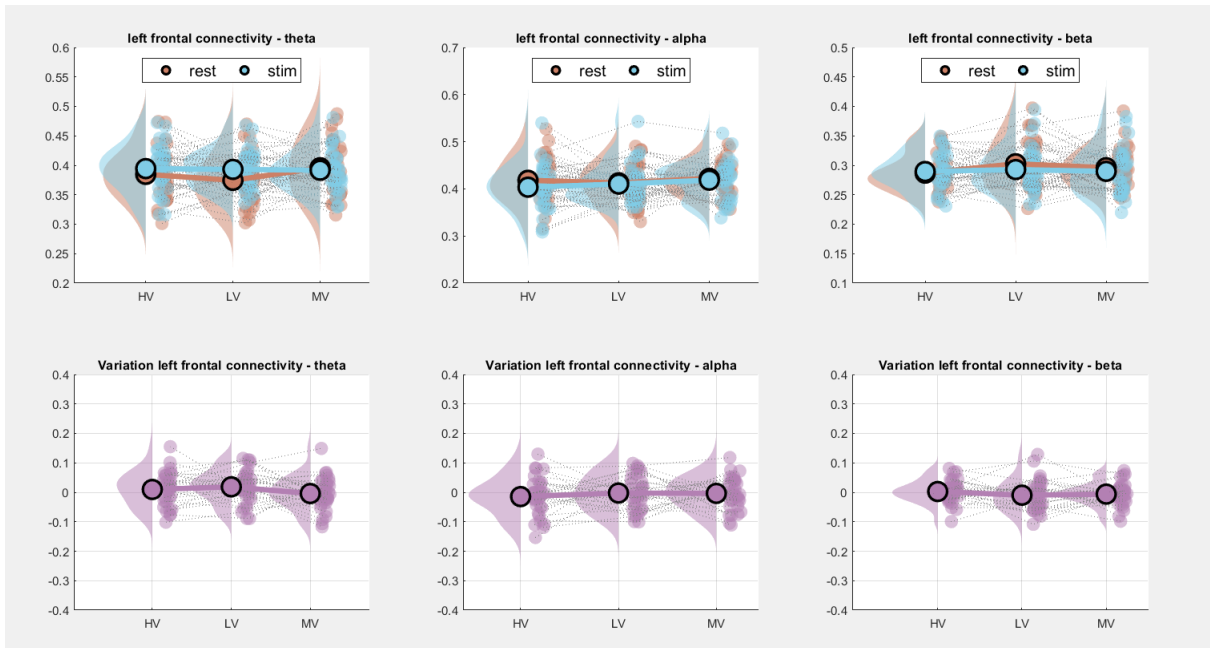


Figure 11. Raincloud plot of the average connectivity values on the left hemisphere for each band, valence and rest and stimuli state. Source: Matlab

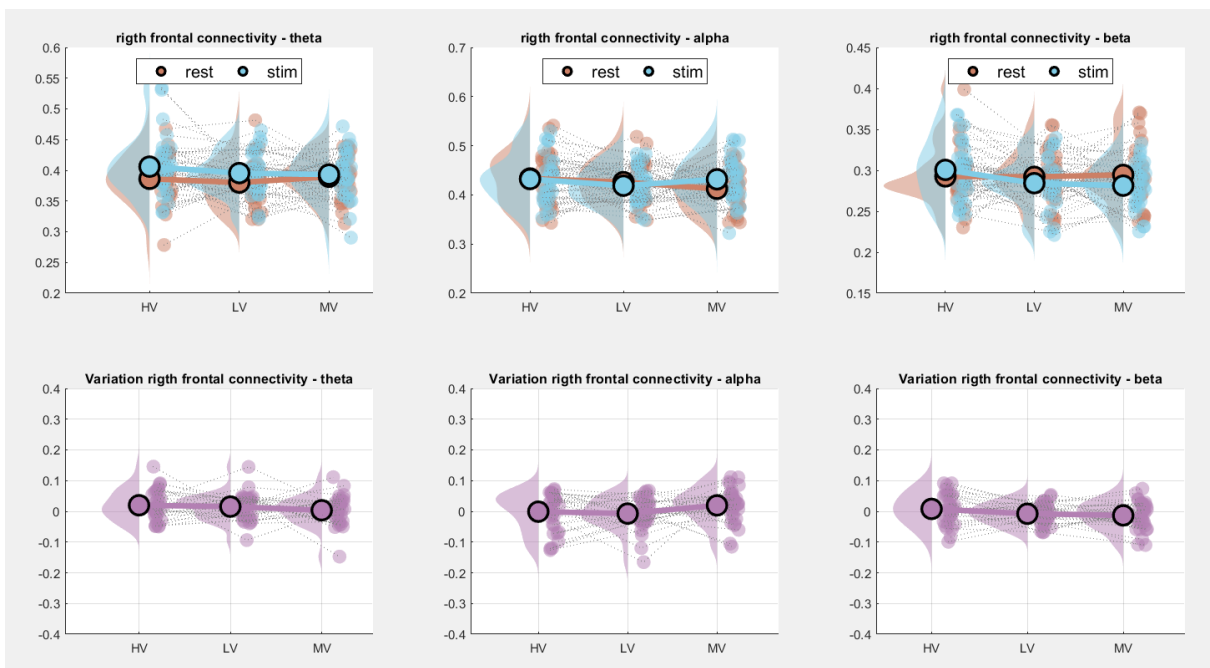


Figure 12. Raincloud plot of the average connectivity values on the right hemisphere for each band, valence and rest and stimuli state. Source: Matlab

As we can see, there are not significant differences regarding the connectivity values between the left and right hemisphere, between the different emotional valences or between rest and stimulation states.

We created the same plots for the graph measures previously calculated. For example, here we can see the raincloud plot for the clustering coefficient:

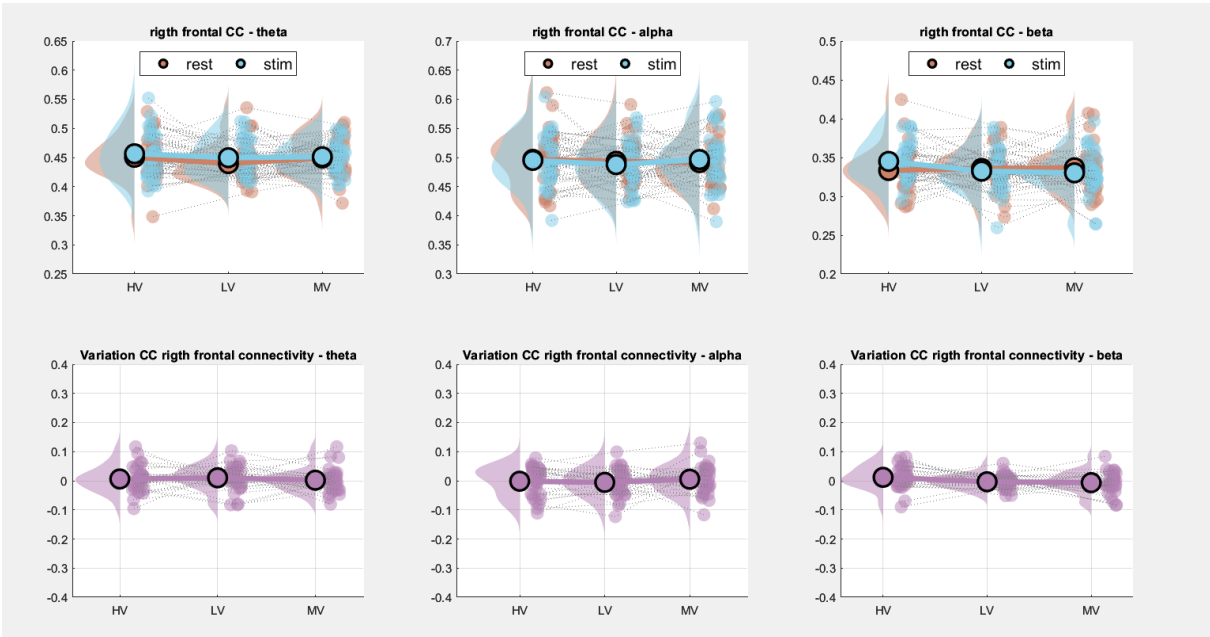


Figure 13. Raincloud plot of the average clustering coefficient values on the left hemisphere for each band, valence and rest and stimuli state. Source: Matlab

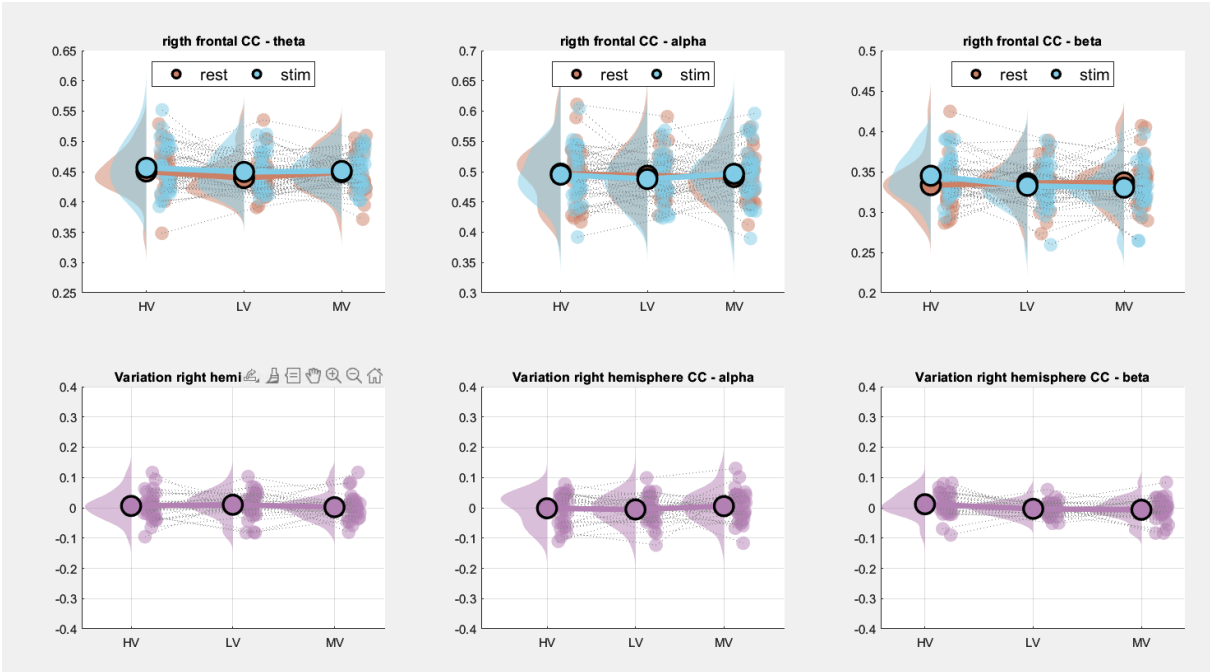


Figure 14. Raincloud plot of the average clustering coefficient values on the right hemisphere for each band, valence and rest and stimuli state. Source: Matlab

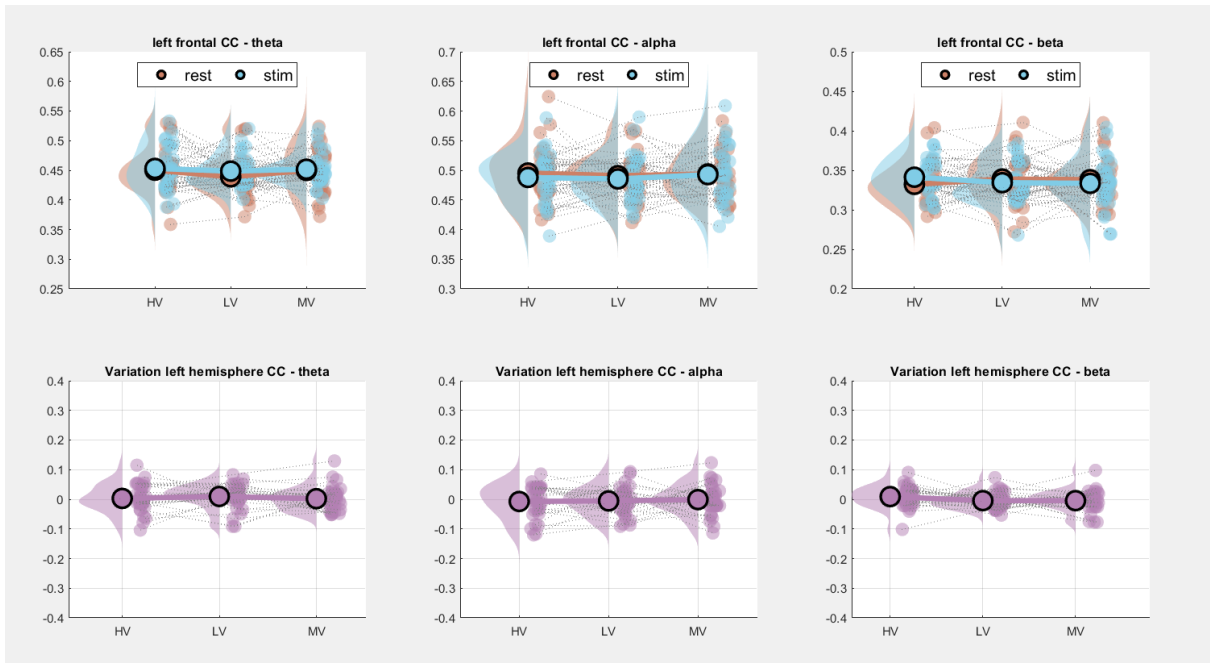


Figure 15. Raincloud plot of the average clustering coefficient values on the right hemisphere for each band, valence and rest and stimuli state. Source: Matlab

We can see that there are not clear differences between emotional valences and rest and stimuli states and neither between hemispheres. We repeated these representations for the rest of the measures and the results obtained were almost the same.

Finally, the last graphical analysis carried out included different boxplot diagrams reflecting the distributions of different measurements under the different conditions. The first one reflects the distribution of functional connectivity values for the different hemispheres and for the different valence, resting and stimulus states. An example of these representations is shown below:

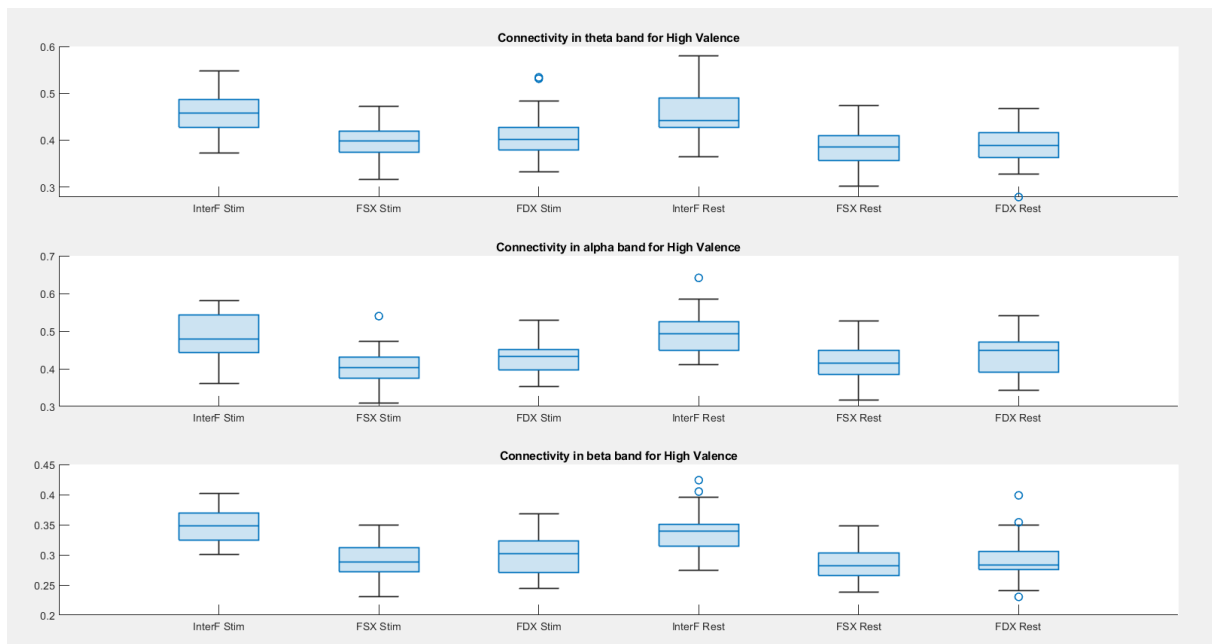


Figure 16. Boxplots of the distribution of the average connectivity values for High Valence state under different conditions. Source: Matlab

We did the same but for the graph measures average values (clustering coefficient, eigenvector centrality, node betweenness centrality and node strength). Here we show an example of the distribution of the node strength in the brain left side.

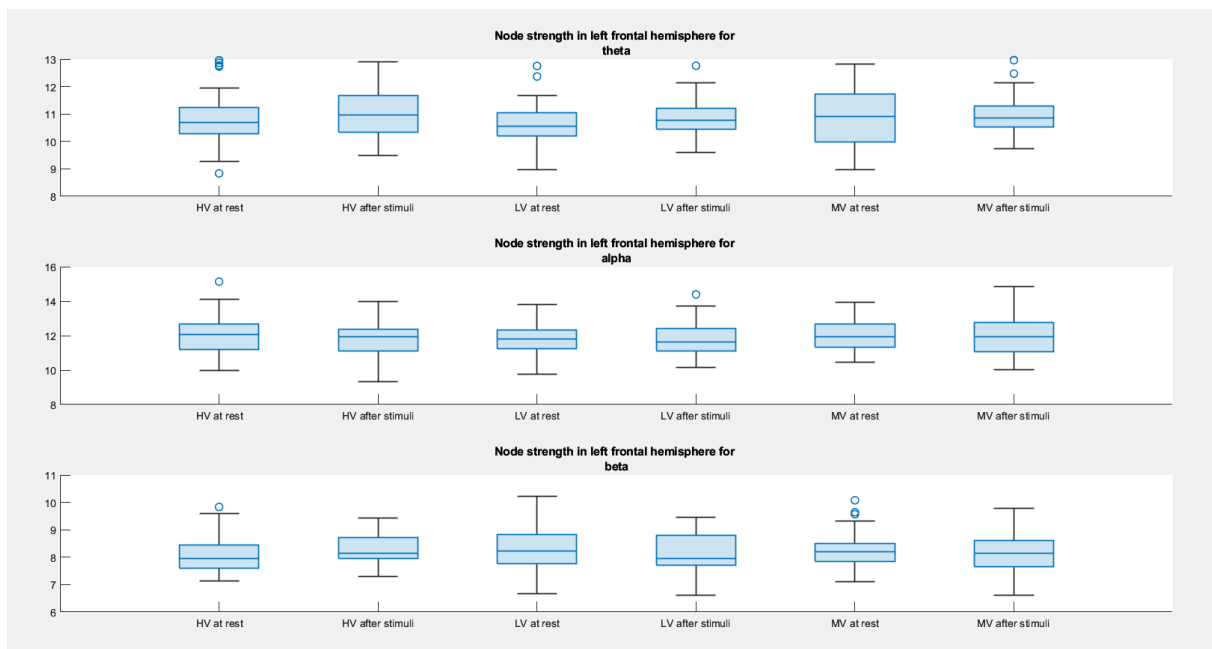


Figure 17. Boxplots of the distribution of the average node strength values in the left hemisphere under different conditions. Source: Matlab

Again, no significant patterns that could differentiate the different emotional states or rest and stimulation states were found visually analyzing the different distributions.

To corroborate these results that have been arrived at visually, by analyzing a series of graphs, we performed a repeated measures ANOVA with the two independent variables brain side and valence. The following table shows the results obtained after calculating the p-value for each measure with respect to these two independent variables, as well as their interaction, for each frequency band.

	Functional connectivity sides (p-value)	Functional interconnectivity (p-value)	Clustering Coefficient (p-value)	Eigenvector Centrality (p-value)	Strength (p-value)	
(Intercept): side	0.0758	-	0.0259	0.0297	0.0231	Alpha band ←
(Intercept): valence	0.4140	0.7309	0.7794	0.3489	0.7835	
(Intercept): side:valence	0.2884	-	0.4629	0.4302	0.4547	
(Intercept): side	0.9887	-	0.6972	0.8085	0.7952	Beta band ←
(Intercept): valence	0.1563	0.2121	0.1176	0.5493	0.1194	
(Intercept): side:valence	0.5656	-	0.4890	0.5513	0.5118	
(Intercept): side	0.4369	-	0.5853	0.7853	0.6096	Theta Band ←
(Intercept): valence	0.1475	0.9806	0.7278	0.7189	0.7378	
(Intercept): side:valence	0.6945	-	0.8154	0.8312	0.8289	

Figure 18. Boxplots of the distribution of the average node strength values in the left hemisphere under different conditions. Source: Matlab

We can see that the only significant values are those indicated in red. That is, we can only establish a significant relationship between the clustering coefficient, eigenvector centrality and node strength measures and the brain side, for the alpha frequency band. On the other hand, there is no significant relationship between the different measures and emotional valence for any of the frequency bands (nor between the measures and the interaction of valence and the cerebral hemisphere).

4.2. Result discussion.

After visually analyzing all the graphs presented, and also analyzing the results of the ANOVA statistical study, we can affirm, with little probability of being wrong, that there is a relationship between the graph measures taken and the brain hemisphere (for the alpha band). The values are higher for the right side of the brain. Despite this, as we have seen, we could not find any relationship or different pattern between the rest and stimulus states nor between the different emotional valences and the measures we took. Therefore, we conclude that the measures selected are not useful for differentiating emotional states, which was the main objective of the study.

5. Conclusions and future work.

The results of the study did not reveal any significant associations between these measures and emotional states. These findings suggest that the examined graph theory measures may not be directly influenced by different emotional states in the context of this study's design and sample size.

We need to understand that, as every scientific research, our study can have limitations that can affect the results we get. Some possible study limitations are:

- Relatively small sample size, particularly in the female group, which may have limited the statistical power to detect significant associations.
- There may be other relevant metrics or connectivity aspects that could contribute to understanding the relationship between emotions and brain connectivity.
- Relying on EEG signals alone may overlook information.

Having these limitations in mind, some recommendations for future research regarding this topic are:

- Future research with a larger and more balanced sample size across genders could provide a more comprehensive understanding of the relationship between connectivity measures and emotional states.
- Future investigations could explore alternative measures such as coherence or mutual information and explore additional aspects of graph theory to further elucidate this relationship.
- We shouldn't rely on EEG analysis alone. For example, we could include multifunctional imaging techniques such as functional magnetic resonance, that can provide a more comprehensive picture of brain networks involved in emotional responses, and of its functioning.

We conclude that, while selected connectivity analysis methods and metrics were not effective in distinguishing or detecting different emotional states, this project contributes to the growing field of emotion assessment through EEG analysis, using connectivity measures and graph theory, highlighting the complexity of emotion representation in brain connectivity and the need for further exploration, using alternative methodologies and measures. Continued efforts in this field are needed to enhance our understanding of the complex interplay between brain function and emotional experiences.

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