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Master's Degree in Biomedical Engineering

Disorder of consciousness evaluation with functional connectivity indexes applied to EEG signals

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A te, Mamma.

Avrei dato la mia vita per salvare la tua

ma ti ringrazio per averla data a me in principio

e per avermi insegnato come viverla.

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Abbrevation	Meaning	Page
A.I.	Artificial Intelligence	68
AMI	Auto Mutual Information	69
ApEn	Approximate Entropy	46
ARV	Averaged Rectified Value	85
ASD	Autism Spectrum Disorder	44
BCI	Brain Computer Interface	43
BCT	Brain Connectivity Toolbox	90
CC	Clustering Coefficient	73
CFC	Cross-Frequency Coupling	52
CMI	Cross Mutual Information	69
CMRR	Common Mode Rejection Ratio	45
CNS	Central Nervous System	11
DOC	Disorder of Consciousness	25
EAP	Extracellular Action Potential	28
ECoG	ElectroCorticoGraphy	28
EEG	ElectroEncephaloGraphy	11
Eglobal	Global Efficiency	73
Elocal	Local Efficiency	74
EPSP	Excitatory Post-Synaptic Potential	34
ESC	Envelope-to-Signal Correlation	53
FC	Functional Connectivity	11
GABA	Gamma-Amino-Butyric Acid	33
GLM	Generalized Linear Modelling	53
GW	Global Workspace	24
iEEG	Intracranial EEG	28
IIT	Integrated Information Theory	24

IPSP	Inhibitory Post-Synaptic Potential	34
KL	Kullback-Lieber	53
LFP	Local Field Potential	28
MDF	Median Frequency	85
MEA	MicroElectrode Array	28
MEG	MagnetoEnchephaloGraphy	28
MI	Mutual Information	68
MNF	Mean Frequency	85
MVL	Mean-Vector Length	53
NCC	Neural Network of Consciousness	23
NN	Neural Networks	68
PAC	Phase-Amplitude Coupling	52
PL	Path Length	73
PLI	Phase Lag Index	70
PLV	Phase-Locking Value	53
PNS	Peripheral Nervous System	11
PSD	Power Spectral Density	84
PVT	ParaVentricular Thalamus	23
RMS	Root Mean Square	85
SampEn	Sample Entropy	48
ShEn	Shannon’s Entropy	85
SNR	Signal-to-Noise Ratio	72
SVM	Support Vector Machines	68
SWS	Slow Wave Sleep	25

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Introduction

Consciousness is an active process with multiple components ranging through different fields of study. These components are closely related and sometimes one can affect the others. One might talk of the blending of different aspects such as wakefulness, awareness, perception, alertness, cognition, and sensation [1].

Generally, the term Consciousness means something different depending on the field of interest:

• *Neurology*

In neurology, consciousness denotes the state opposed to coma. There are different stages of consciousness that can be distinguished starting from full consciousness, ranging to intermediate states in which a person detains some of their own perception capabilities, up to a total consciousness absence, and these variations occur due to severe physical brain injuries. These can have a traumatic or not traumatic etiology and bring to a coma, a vegetative or semi-awake state. The results are classified according to the level of wakefulness and awareness, and following the needed recovery time, if there is one.

• *Psychiatry*

In a psychopathological sense, it's the capability to discern Io from the outside. It defines a mental disorder as a syndrome characterized by a clinically significant disturbance in an individual's cognition, emotion regulation, or behavior, and reflecting a dysfunction in the psychological, biological, or developmental processes underlying mental functioning. We are talking about dissociative identity disorder, dissociative amnesia, depersonalization/derealization disorder. We encounter consciousness disturbances in patients with impaired memory, executive functions, perception, insight and monitoring. Alienation in every day's life and out-spacing might lead to psychosomatic symptoms, like panic attacks, labile sleep-wake cycle, reality testing and cognitive failures.

• *Ethics*

On an ethic aspect, it's the capability to discern the good from the evil and act accordingly. It represents a sort of inner instinct that tells us what is right and how to decide that something is right. It affects our decision making, basing every practical action on education, experience, and information. It can be viewed as a moral muscle because it is not innate and is continuously strengthen according to our surroundings.

Even if they might not look tightly related, all these aspects of consciousness are proven to be linked: morality becomes altered when a psychopathological disorder occurs, and both neurological and psychic impairments can be reconnected to trauma of the brain, especially in particular zones.

The main goal of this study is to reach a better understanding of the neurological aspects that affect consciousness in several injured patients, according to the stages mentioned above.

Nevertheless, nothing prevents our results from being expanded to other branches of brain analysis, regarding both other scopes and conditions.

We will start with an overview of the brain's anatomical and functional structure, focusing on the aspects related to the object of interest, i.e. consciousness and its loss. Once the main problem's characteristics and causes are presented, we will move on to comprehend the major means used to study the phenomena, referring to analysis made on the brain signal. Electroencephalographic (EEG) signal is therefore discussed, along with all its specifications and possible applications, followed by an explanation of how these signals could be treated in order to understand and classify consciousness diseases.

Applied to EEG signals, **Functional Connectivity** (FC) is then introduced as the main means of analysis, along with Graph theory on which it is based and Connectivity Indexes.

Entropy and **Cross-Frequency correlations** are also presented as additional means to investigate statistical and predictive patterns in different time-series or frequency bands from different brain sections.

Similar projects are presented and analyzed to compare different pathologies' behaviors and different techniques used to study them.

In the end, the algorithm and the results on the work subjects are presented, along with a discussion on our successes, limits and future thoughts.

Chapter 1

Nervous System

1.1 Introduction

The main distinction in the nervous system is between the central and peripheral systems.

The **Central Nervous Syste**m (CNS) is composed of the brain and the spinal cord. It receives all the information which then get processed in order to decide what to do and which area should act and how.

The **Peripheral Nervous System** (PNS) consists of motor nerves and sensory nerves which branch from the spinal cord and from the brain. They bring information in and out of the CNS [2].

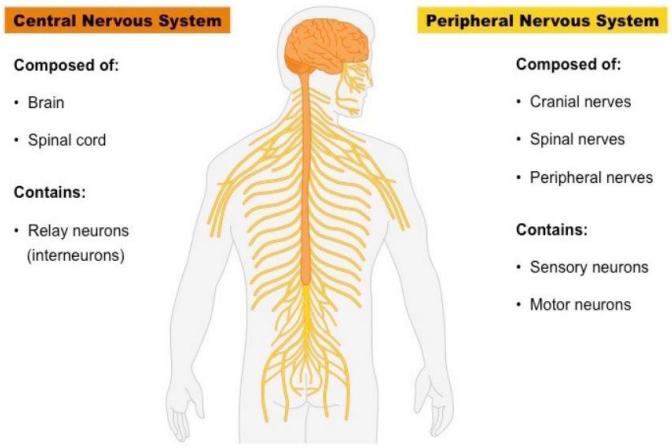


***Figure 1**. Central Nervous System (CNS) and Peripheral Nervous System (PNS) composition [2].*

1.2 Brain

The brain is the central organ of the nervous system in humans and most animals. It is a complex organ with a large number of functions, ranging from thought control, memory, emotion, motor skills, vision, breathing, regulatory processes.

It contains blood vessels and nerves, including neurons and glial cells. The human brain weighs around 1.2 - 1.3 Kg in adults and is mainly made of fat, water, proteins, carbohydrates, and salts. Neurons are the most important type of cells in the brain. There are approximately 100-150 billion neurons, and each neuron has 10^3-10^4 connections. They communicate with other areas using axons

which transmit signals to other neurons by junctions named synapses. The transmitted signal is called action potential and when it gets to a synapse a neurotransmitter is released which link to the target cell. Glial cells, on the other hand, serve as structural and metabolic support, protection, and insulation for the neurons [3].

The human brain is subdivided into:

- Cerebrum
- Brainstem
- Cerebellum
- Diencephalon

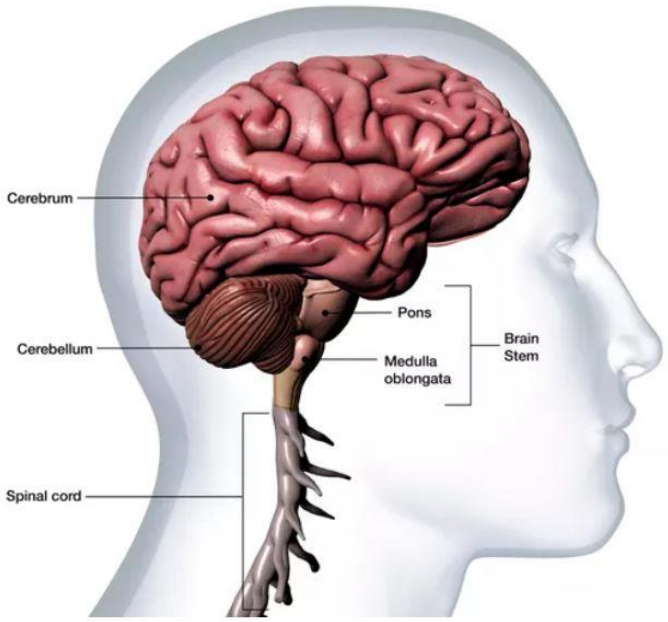


***Figure 2**. Human brain main parts: cerebrum, brain stem, cerebellum, spinal cord, pons and medulla oblongata [3].*

1.3 Cerebrum

The **Cerebrum** is the largest part of the brain and it runs and coordinates movements, speech, thinking, reasoning, emotions, and learning, other than the senses. The Cerebral cortex covers the cerebrum and folds in gyri and sulci in order to extend to a larger area. It's divided into 2 hemispheres joining in a deep sulcus called interhemispheric or medial longitudinal fissure which runs from the front to the back of the head. Right and left hemispheres control the opposite side of the body, and they communicate through the so-called corpus callus, a structure with a shape of a letter C located in the center of the cerebrum made of white matter and nerve pathways. The cerebral hemispheres contain a lateral ventricle filled with cerebrospinal fluid. The superficial layer of the cerebral cortex is composed of gray matter, neuronal cell bodies and unmyelinated axons, while the inner area is made of myelinated axons, known as white matter. Basal nuclei are in the white matter and consist

of neurons involved in functions such as interpreting senses, speech, reasoning, learning and motion control.

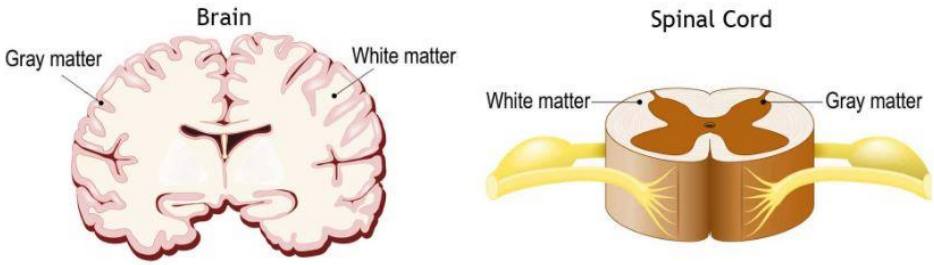


***Figure 3**. White and Grey Matter's disposition in the Brain and in the Spinal Cord [4].*

Three different fibers groups can be distinguished:

- associational fibers, which coordinate areas within the same hemisphere;

- commissural fibers, to connect the two hemispheres in corpus callosum;

- projection fibers, forming ascending and descending paths that connect thalamus to the cerebrum and cerebellum to the spinal cord [4] [5].

1.4 Cerebral cortex

The **cerebral cortex** is composed of bands of gray matter which set its functional units. They are vertically arranged and separated into six layers.

A characteristic distribution of neuronal cell types and connections with other cortical regions is found in each of the cortical layers. The layers contain two nerve cells type: excitatory and inhibitory.

Excitatory neurons represent the majority of the nerve cells, around 70-80%. They can be of two types: pyramidal cells and spiny stellate cells. The former have dendrites which collects inputs from other neurons across all the layers. The latter, instead, have short dendrites and are mainly found in layer IV as local neurons.

Inhibitory neurons, on the other hand, represent the remainder of the cells in the neocortex. Their dendrites receive both excitatory and inhibitory synapses, and they get through the white matter. Those neurons make gamma-aminobutyric acid (GABA) at their synapses. Cortical cells receive activity from many afferent neurons and the of the present connections, some are excitatory and others inhibitory, but it is to be noted that the impulses can activate a large number of cortical neurons in many patterns of excitability [6].

The six layers of the cerebral cortex are:

- **Layer I**: Molecular layer
  It contains few neurons and is composed of dendrites coming from pyramidal neurons in deeper layers and horizontally running axons. Impulses arrive to the dendrites from the thalamus and the brain stem.

- **Layer II**: External granular layer.
  Contains stellate cells and small pyramidal cells, with impulses coming from other parts of the cortex like callosal and association fibers.

- **Layer III**: External pyramidal layer.
  Contains small to medium-size pyramidal cells. It's the main source of interconnecting fibers for the different areas of the cortex.

- **Layer IV**: Internal granular layer.
  It contains many non-pyramidal neurons and receives much of the sensory input coming TO the cortex (Afferent fibers) from the thalamus and from callosal and association fibers.

- **Layer V**: Internal pyramidal layer.
  It contains the largest pyramidal cells and long axons that project OUT of the cerebrum (Efferent fibers) to the spinal cord and the brain stem.

- **Layer VI**: multiform layer.
  It consists of the maximum variety of cell types which reach the thalamus and the rest of the column [7] [8].

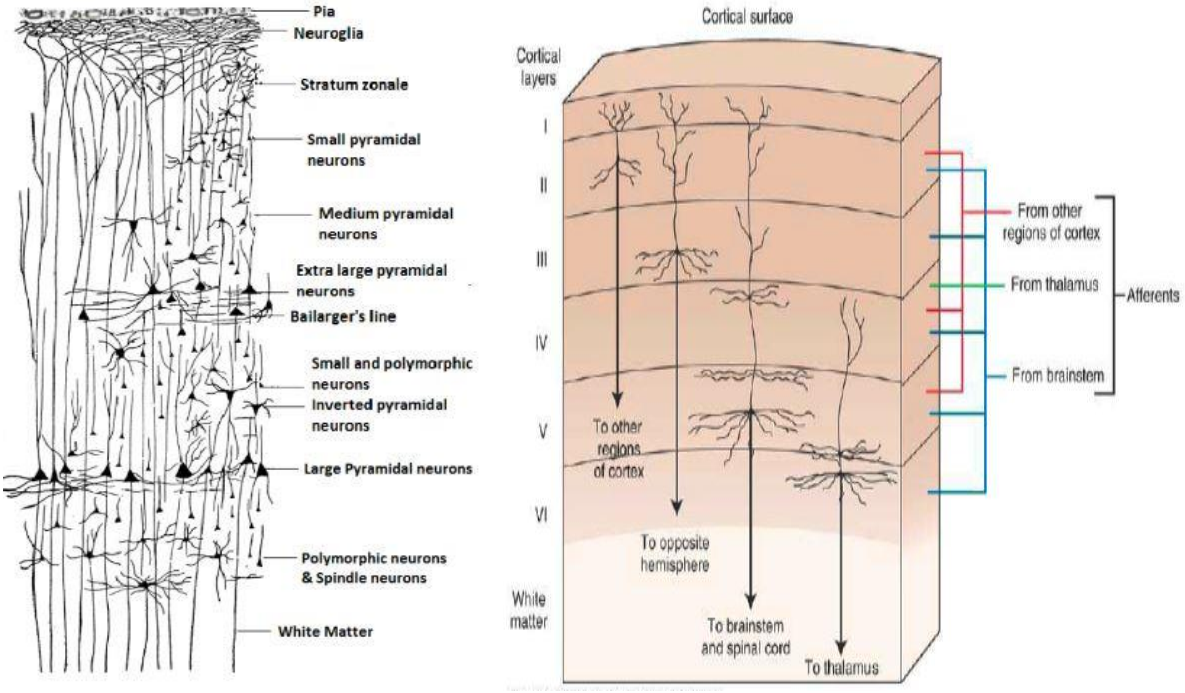


Figure 4. A) Histological Structure of the cerebral cortex showing the distribution of neuron types across the layers; B) Function and connection of every layer of the cortex, both afferent and efferent [6] [7] [8].

Corpus callosum

It's a nerve fibers mass which links the two cerebral hemispheres. The commissural fibers are located here.

Meninges

Surrounding the brain, we can find different protective layers. From the exterior to the inside, we can find: Scalp (skin), aponeurosis, periosteum, skull (bone) and then the meninges.

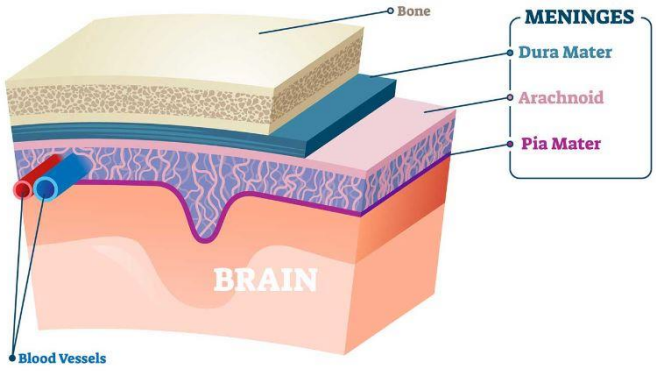


***Figure 5**. Order in which the meninges are arranged between the skull and the brain: Dura Mater, Arachnoid, Pia Mater [4].*

The meninges are membranes directly surrounding the brain and the spinal cord:

- The external one is the dura mater, thick and tough, made of fibrous connective tissue. It lines just below the skull has spaces to allow blood vessels to pass in order to supply blood to the brain.
- The middle layer is the arachnoid mater, thin and web-like, made of connective tissue with no nerves or blood vessels. Below it lies the cerebrospinal fluid which dampens and cushions the whole CNS also removing all impurities.
- The inner layer is the pia mater, a thin membrane that follows the brain's contours. It is rich of arteries and veins [4].

1.5 Functional areas of the cerebral cortex

Association areas

There are three main association areas in the brain: premotor, somatosensory, and visual. Not all movements or sensations are necessarily connected to the same areas of the brain, so it might be required to integrate all kind of information.

Functional areas: Cerebral Cortex Lobes

The brain is composed of 2 cerebral hemispheres (right one and left one) and each one has four mapped sections, called lobes: frontal, parietal, occipital, and temporal lobes. Each lobe has its own functions and roles, even if they are interconnected.

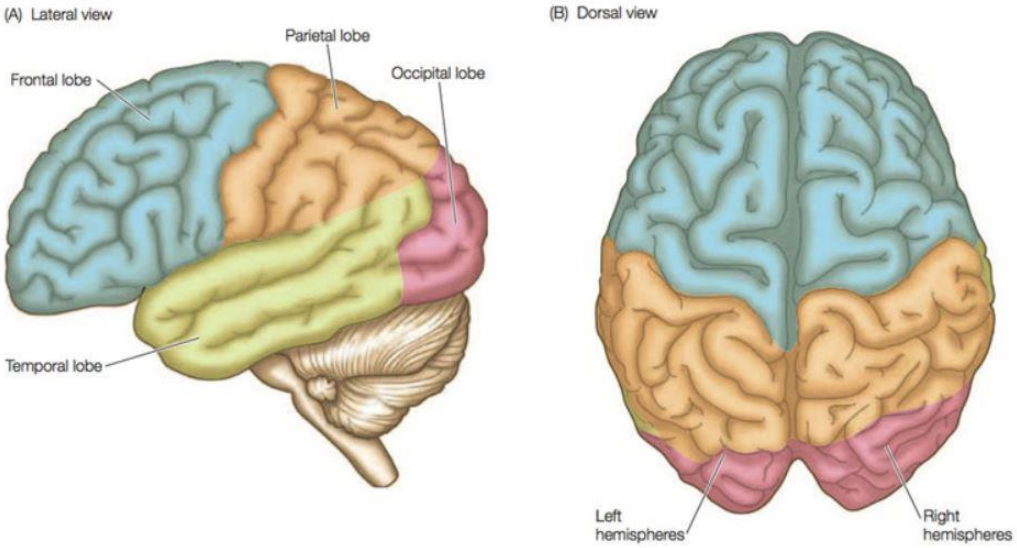


***Figure 6**. A) Lateral view of the brain showing the four mapped lobes: Frontal, Parietal, Temporal and Occipital Lobe; B) Dorsal view of the brain showing the Left and Right Hemispheres, divided by the central sulcus [9].*

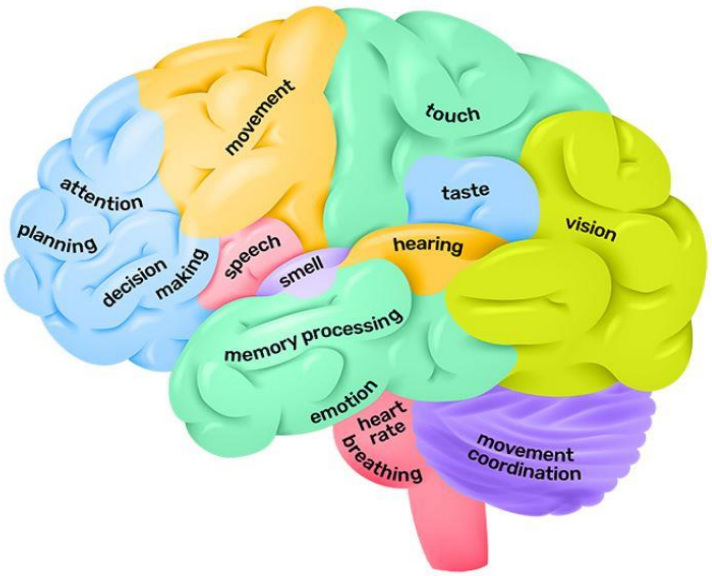


***Figure 7**. Subdivision of the brain according to the main function of each section [11].*

- **Frontal lobe**: it is the largest of the brain. It is located in the front of the head in the anterior portion of both the hemispheres. It concerns the primary motor cortex and the generation of descending impulses involved in producing voluntary movements. It is responsible for determining personality characteristics and emotional responses, decision-making, part of

recognition of smell and speech ability. This last one is due to the Broca's area situated right in the frontal lobe, and includes verbal fluency, phonological and grammar processing, and speech attention.

- **Parietal lobe**: located right behind the frontal lobe and superior to the temporal and occipital lobes. It stands out for the somatosensory cortex. In fact, it receives sensory information from all sensory receptors that provide information related to temperature, pain, proprioception and fine touch. It leads to understanding space and objects of the environment, and spoken language thanks to the Wernicke's area.

- **Occipital lobe**: located in the most posterior portion of the brain, behind and below the parietal lobe and connected to the optic nerves. Here visual processes take place receiving impulses from the retinas. The main visual functions consist of color determination, depth perception, visuospatial processing and even some memory formation. Visual perception is accompanied to visual information interpretation.

- **Temporal lobe**: on the sides of the brain, beneath the frontal and parietal lobe. Its cortical areas are involved in the auditory and olfactory functions thank to the ears and nasal receptors. It also manages short-term memory, speech, and musical rhythm. It also contains the Fusiform Face Area (FFA), necessary for face recognition.

In addition, in a deeper section of the brain one can find the Insular Lobe and the Limbic Lobe, which are responsible for processing and integrating various kinds of information such as taste, visceral pain and vestibular sensations, other than neuropathic pain and nociception [10] [11].

1.6 Brain stem

The brain stem connects the spinal cord to the cerebrum and consists of midbrain, pons, and medulla oblongata. It contains nerve fibers which descend and ascend from the spinal cord and collections of neurons for motor and sensory processes.

Midbrain: also called mesencephalon, it is a structure with a certain number of neuron complexes and pathways suited for functions such as hearing, movement, responses to the environment. It extends between the hypothalamus and the pons. Substantia nigra lies inside the midbrain, enabling coordination in movement, and is affected when Parkinson's disease occurs. Among its functions we find eye and body movement, and muscle reflexes.

Pons: it represents the link between medulla and midbrain, like a bridge, and it is where some cranial nerves generate. These enable different activities like balance, expression, blinking, producing tears, focusing during vision.

Medulla: it lies at the end of the brain stem, superior to the spinal cord and in front of the cerebellum linking the brain to the spinal cord. It regulates heart rhythm, breathing, blood flow and oxygenation level. It is also responsible for physiological reflexes such as coughing, swallowing, vomiting, and sneezing.

The spinal cord spreads from the bottom of the skull down to the vertebrae which support it. It brings all kind of information from and to the brain and the whole body [1] [4].

1.7 Cerebellum

The Cerebellum is a quite small portion of the brain located behind the pons, and above the brain stem to which it is connected. It has two hemispheres, like the cerebral cortex, so it is a bilateral structure. The outer portion contains neurons while the inner one communicates with the cerebral cortex. Although it has no direct connection to the spinal cord, it manages to influence movement through the connections to the cerebrum and the brainstem. Its main role is to control voluntary muscle movements, posture, balancing and giving feedback information to the motor coordination and eye movement. It plays a role also in social behavior, emotion, addiction, and even schizophrenic actions. When disorders of the cerebellum occur, a lack in movements coordination might appear, known as ataxia [3] [4].

1.8 Diencephalon

The Diencephalon is located ahead of the midbrain and consists of the Pituitary gland, the hypothalamus and the thalamus, the amygdala, the Pineal gland, and the hippocampus.

The **Pituitary gland**, or hypophysis, is a tiny structure that operates over the other glands of the body, with a hormonal regulation function. It gathers information from the Hypothalamus, which is located just on top of the pituitary gland.

The **Hypothalamus** deals with homeothermy, sleep patterns, hunger and thirst, and some aspects of emotions and memory. It represents the main connection between the nervous system and the endocrine one.

Above the hypothalamus but inferior to the cerebrum is located the **Thalamus**. Its functions relate to sensations due to various impulses. The information is here gathered and sent to the cerebral cortex where the sensation is then processed. It distributes the sensorial information to the right region of the cerebral cortex.

The **Amygdala** is a structure found under both hemispheres of the brain. It regulates emotions associated with stress, threats, and fear.

The **Pineal gland** is located very deep inside the brain and its functions lie in the response to light and dark, and the regulation of the sleep-wake cycle and the circadian rhythms.

The **Hippocampus** is an organ which resembles a seahorse in its shape. It is located below both the temporal lobes, as there are two, and it plays an important role in supporting short-term and long-term memory, spatial memory, and perception. It receives information from the cerebral cortex, and it is one of the first organs to suffer from the Alzheimer's disease, bringing to memory loss and disorientation [4] [5] [7].

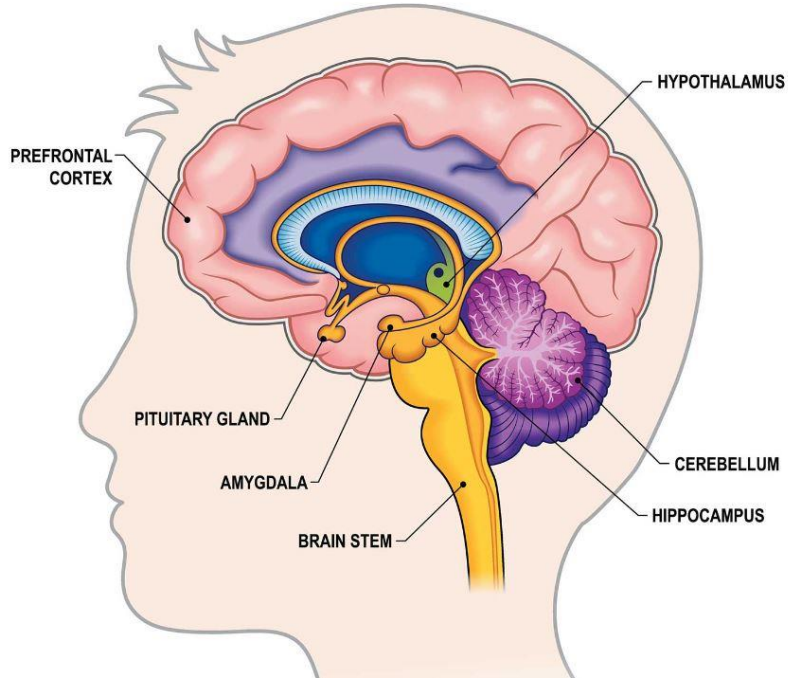


Figure 8. *Dienchephalon's position according to other structures and Diencephalon's structure which includes Hypothalamus, Thalamus, Pituitary Gland, Amygdala, Hippocampus [4].*

Chapter 2

Consciousness

2.1 Consciousness

From an anatomical-functional point of view, psychologist and philosopher William James defined consciousness as a state of a being aware of an environment and of self-hood. This definition is based on complex brain functions which involve interrelated components. Consciousness is given as an integration of two physiological components: **alertness**, also known as state of consciousness, and **awareness**, or content of consciousness. Both are interconnected, constituted of multiple elements.

- The former is controlled by the ascending reticular activation system consisting of a series of nuclei present in the upper brain stem tegmentum and midline and intralaminar nuclei of the thalamus. The activation structures are not confined in the encephalic trunk and their influence reaches the spinal cord and the cerebral hemispheres. The thalamic reticular nucleus is responsible for selection specific reticular information transmitted to the cerebral cortex, providing feedbacks to the brainstem centers which arouses alertness. This selection is central for attention and concentration. In addition, the hypothalamus plays a crucial rule in sleep induction, controlling sleep-wake cycles.

On a physiological level, alertness is characterized by a series of chain events which start with the brain stem activation and end with the cerebral cortex activation due to the thalamus. In particular, it is the pallium that has the function of processing all the sensational information and generating the experience of consciousness. From a neurological point of view, alertness requires more attention as a disorder of this state might lead to coma or a vegetative state

- The latter sums up cognitive and emotional functions relative to physiology and anatomy of perception, attention and emotion centers in the cerebral cortex.
  Perception and sensation are interdependent activities:

    - Sensation involves individual awareness, like a result of something happening. Visual, auditory, somatosensory, olfactory, gustatory, vestibular and visceral sensations derive from primary cerebral association regions, and they have spatial and temporal location. The involved regions are interconnected and two-way systems. Both serial and parallel processing of sensations occur. In addition, sensations are connected to memory as this allows further experience in feeling.

    - Perception instead involves external happening awareness and it overrides sensation when they both occur. From a visual point of view, perception allows to differentiate background from a subject, shapes, object recognition.

Attention is part of perception, but it needs both wake and alertness. It takes in account selection and concentration on processing information exclusive to some stimuli other than others. Choosing which information needs to be considered or not is a task performed by the prefrontal region with further processing and modulation in memory and motivational areas.

Memory, on the other hand is composed of distinct parts, included in consciousness. It is said that memory and learning take place whenever a certain axon of a cell is repeatedly taking part in firing to a certain other cell, until it enhances its efficiency in firing, growing a better connection between the two cells. Explicit memory recalls related to bindings of neural events by the hippocampus are usually associated to consciousness, which gives sense of familiarity and continuity to a certain memory or moment.

Consciousness also involves the motivational system as it is related to perception, aimed activities, emotional cognition, motivation. These entities are all gathered in neurotransmitter system network and thus can't be isolated. The amygdala, hypothalamus and limbic structures are all responsible of feelings and motivation. The hippocampus and the amygdala receive projections from the sensory systems, but the hippocampus consolidates information into memory, while the amygdala gives sensory and affective to the experience, expressing an emotion thanks to the hypothalamus.

Cognition is strictly related to consciousness, as the latter's components activity results in the processing of the former. It consists of self-awareness, self-esteem, distinctiveness from others. Language is also a highly developed cognitive activity due to the temporo-parietal region.

Kinsbourne defined cognition through a model made of a network of connected regions of cerebral cortex. In this system, the most active neuronal module achieves consciousness at a time, like as there were phenomena which could override others such as pain.

Moscovitch claims that memory functions are integrated into cognitive activity such as monitoring, self-organization and strategic intervention. All these would be verified in the frontal lobes and then the prefrontal regions would provide further planning of action [12] [13].

2.2 Consciousness' main functions

Consciousness practices a lot of vital and interconnected functions in the CNS which can be summed up as:

- Definitional and Context-setting Function
- Adaptation: in a new context or event
- Learning: from new events and experience
- Editing, Flagging, and Debugging: to modify and change errors
- Recruiting and Control Functions: to control physical and mental action through the recruiting of intermediate goals
- Prioritizing: focusing attention to certain mechanisms or content

- Access-Control: control over what will become conscious

- Decision-making or Executive Function: consciously resolve a choice when automatic systems encounter something which is not routine

- Analogy-forming Function: associating content to known information in order to access possible inputs to new information

- Metacognitive and Self-monitoring Function: to think about consciousness and control its functioning

- Auto-programming and Self-maintenance Function: stability is maintained providing information though conscious experience [1].

2.3 Consciousness key nuclei

Paraventricular nucleus

Paraventricular nucleus is a neurosecretory nucleus of the hypothalamus associated with functions such as hunger and behavioral control, but also sleep promotion and arousal through production of hormones like orexin. Orexin-activated paraventricular thalamus (PVT) neurons operate upon sleep-wakefulness, so we could conclude that the paraventricular nucleus of the thalamus would be involved in arousal control. It was demonstrated indeed that PVT shows high activity during wake and inhibition in a decreased arousal state.

Claustrum

Claustrum is an irregular sheet of neurons located in the center of the brain, under the neocortex. It is linked to the various areas and lobes of the brain, suggesting a gateway role for perceptual information and arousal system. It has been proven that the claustrum works for consciousness restoration but not for its maintenance.

Neural Network of consciousness

The various consciousness key nuclei and generation areas can be described from a neural mechanism which produces the least consciousness called Neural Network of Consciousness (NCC). The NCC would be distributed in all parts of the brain and would be coordinated to function in collaboration. In a neural network, paraventricular nucleus controls arousal, maintaining consciousness, while the claustrum is related to integration and generation of conscious information.

Vital cortical region regarding consciousness

Activation of the brain has been observed mainly near the central sulcus or in the posterior area, rather than the prefrontal cortex. People who endure back brain lesions or resections tend to be more frequently involved in persistent vegetative state while patients result to remain conscious even after bilateral frontal lobe resections, indicating this area as not essential for consciousness [12] [13].

2.4 The study of consciousness

Consciousness can be studied in different ways, and for different reasons. Wakefulness can be better quantified and observed unlike awareness which is more difficult to record. Proceeding step by step, one might start analyzing the moment during which a person gains consciousness, that is awakening. The opposite state to wake is coma, and one could study the intermediate states which could occur from one extreme to the other.

Wakefulness shows up differently referred to coma or sleep. There are clear distinctions made in their characteristics, ranging from missing eyes opening, possibility to wake up or semi-wake.

Theories about consciousness

Interpreting consciousness in a precise way is complicated, and we may find different approaches based on the studies of psychologists and cognitive scientists who tried to create neuroanatomical and neurophysiological models of the brain:

- **Global Workspace Theory**

  The Global Workspace (GW) theory was proposed by Baars and further developed. Baars suggested the competition of parallel, coexisting processes which could be selected and achieve consciousness. Various processes could be based on a starting conscious cognitive content. All regions of the brain join and share participation and coordination in order to accomplish the generation of a conscious experience.

- **Integrated Information Theory**

  The Integrated Information Theory (IIT) is based on Tononi's work. He derived his contribution starting from a conscious result and going backwards, trying to understand the reasons behind. Consciousness, in this case, is the capability of a system to integrate information, and it is based on an internal causal power which determines quantity and quality of an experience [12] [13].

Chapter 3

Disorders of Consciousness

3.1 Brain damage

The human brain is certainly susceptible to many types of disease and damage. Infections to the brain might be rare as it is protected by blood and fluid barriers. Even though the brain is protected by the skull bones, cerebrospinal fluid suspension and bloodstream barrier isolation, physical damages happen more frequently due to head injuries and trauma, strokes or even chemical poisoning. In this case, the different brain structures might stop communicating and collaborating, which could lead to incomplete or misfunctioning tasks.

One of the possible consequences of these brain damages is a certain grade of consciousness loss.

Consciousness represents such a complex notion which goes beyond the simple sum of its different parts. All the more so, consciousness disorder is a too vague term as in any individual case we need to know exactly which part of consciousness is impaired and to what extent [14].

3.2 Consciousness disorders

We can't speak of consciousness as a black or white state, because there are many shades that describe its components. Arousal, in particular, is not only defined as wakefulness, but there are intermediate states between wake and sleep that could be physiological or not. The sleep phases can be analyzed as a physiological phenomenon, while coma, vegetative state or minimally conscious state represent some of the pathological cases indicating a disorder in consciousness. These might occur following a brain injury which could interrupt communication between consciousness' involved structures such as the cerebral cortex, the brainstem or the thalamus. The aetiology of the disorder, though, could be traumatic or not depending on its level and manifesting form. Whenever a serious brain injury occurs, the first two possible scenarios include either death or a coma. The patient suffering the coma might remain unresponsive, evolving in a vegetative state. Afterwards, there's a possibility for the patient to get in a minimally conscious state or persist in a long-term vegetative state. For the minimally conscious state could, consciousness might be restored in a certain number of weeks, while a persistent vegetative state might last years. Locked-in syndrome in another option which implies the patient to be awake but unable of movement functions, except eye movements. It is a debilitating condition that can be tried to get eased using BCIs [1] [14].

3.3 Consciousness disorders classification

Clinically speaking, **Disorders of Consciousness** (DOC) can be classified in an order of increasing severity as:

- **Confusion**. It can be physiological. A confused person might seem disoriented in time and space. Its EEG shows random slow waves with a disorganized background activity.

- **Drowsiness**. A drowsy person might be unable to remain awake without and external stimulus. EEG-wise, it appears similar to light sleep.

- **Stupor**. In this condition, a person results unresponsive to stimuli. Only exploiting nociceptive or other kinds of energic stimuli might be sufficient to temporarily wake a person, but at the end of the stimulation the attention seems to go down again. The EEG in this case shows high amplitudes in the waves of Delta and Theta frequency range [1].

3.3.1 Reversible unconsciousness

Unconsciousness is a completely normal experience that everyone lives during sleep. In fact, deep sleep is gained through neural modulators every night. Anaesthesia is not a physiological unconscious state, but is it controlled and at low risk for a patient to not regain consciousness afterwards. The brain undergoes processes and functions which differ significantly between deep sleep and sedation.

- **Slow wave sleep** (SWS). During SWS, brain processes are slowed down by hormones and neural modulators, until the deep sleep is reached. Its main characteristic are the EEG slow waves which appear through all the brain, but in specific regions in particular, involving memory and cognitive areas which are silenced. Since the activity is different among the brain's areas, arousal is allowed by external stimuli like noises. SWS automatically progresses to other sleep phases, such as light sleep, rapid eye movement and wake.

- **Anaesthesia**. Anaesthesia is controlled by many substances which differentiates this state from the SWS. Agents for amnesia or memory loss are included, with analgesics for pain relief, reflex suppressors for immobilization and hypnotic state inducer to reduce awareness. Chemically, the anaesthesia reduces the flow of sodium molecules into the neurons, blocking the spread of action potentials. In SWS there is no such brain activity reduction. When necessary, anaesthesia can be reverted by just reducing these substances' flow. Arousal occurs, them, in minutes or hours [14].

3.3.2 Pathological conditions

- **Post-Traumatic Confusional State**. This state follows a trauma like a hit or a fall where the head is involved. It leads to a confusion feeling which extends to many days or more. In this state, extended wakefulness periods occur, while the patient appears clearly disoriented. These

patients are capable of consistent completing commands, employ language functions and recognize objects. This might not be the case of minimally conscious subjects.

- **Minimally Conscious State**. It represents a severe clinical condition in which consciousness is impaired but with some voluntary yet inconsistent behavior that are reproducible and not considered as reflexes. It is a consequence of a coma and a following vegetative state and might last either for a long or short time. It typically represents a transitional state, leading either to the patient's recovery or neurodegeneration. It can't be unequivocally diagnosed as a conscious state, but there are some particularities that might help identifying the right state:

    - sleep-wake cycle presence;
    - communicative skills;
    - purposeful motor activity;
    - eye-tracking.

- **Vegetative state**. It occurs as a clinical condition after a period of coma caused by injuries, ictus or cerebral hypoxia. Self-awareness and of the environment are not present, and response to motor or verbal stimuli is absent as well. Sometimes, intermittent vigilance appears in the form of eye opening in between of the sleep phases. A patient in a vegetative state preserves autonomous functions, surviving with the provision of medical care. In fact, even if the patient lies unconscious needing hydration and nutrition assistance, vital functions support tools are not required. There's no evidence that proves or refutes that vegetative patients could feel some sort of suffering.
  Vegetative state diagnosis requires:

    - Wakefulness absence;
    - No response to stimuli in any voluntary, behavioral or reproducible way;
    - No verbal comprehension and production;
    - No environmental interaction;
    - Ocular motility absence;
    - EEG which testifies Sleep cycles;
    - Autonomous vital functions;
    - Cranial nerves functionality impairment;
    - Rigidity and spasticity.

- **Coma**. Coma occurs as a consequence of a severe brain injury, which could be of a structural or metabolic character, and in a local or diffuse form. The injury usually involves both the hemispheres, the thalamus and the brainstem. With coma, every somatic function like sensitivity, motility, understanding, is missing. Coma differs from the vegetative state because in this case vital functions are affected and could undergo some dysfunctions. Therefore, heart activity, blood pressure and respiration need to be controlled and aided. In addition, no external stimulus elicits arousal, making coma different from a physiological sleep. No sleep-wake cycle is present or measurable by EEG, the eyes remain continuously closed, and no purposeful motor activity appears.

- **Locked-in syndrome**. It is a clinical condition, but it is not a consciousness disorder. Locked-in patients remain alert and wake, with serious motor functions deficiency, making it difficult

to identify real consciousness signs. Locked-in subjects suffer from damage limited to the brainstem, while the cortex is unaffected. The only movement possibility for these patients lies in the vertical eye movement, as they are generally quadriplegic and anarthric. This syndrome may appear in two forms: incomplete or total. The incomplete locked-in syndrome presents an indeed incomplete quadriplegia with the presence of said eye movements; the total form, on the other hand, prevents even the movement of the eyes, making the syndrome a challenge to diagnose. Vegetative patients might have eye openings episodes, but Locked-in patients' eyes' movements present higher levels of wakefulness and awareness. In the right circumstances, this allows to distinguish between the two conditions, avoiding a patient to be insufficiently treated.

- **Blindsight**. It is a consciousness disorder that involves the awareness content, unlike the previous pathological conditions which concerned the state of alertness. People with blindsight are completely unable to see, yet they are paradoxically capable of discerning the environment's features and the objects composing it. This condition derives from damages of the primary visual cortex. In detail, when such injury occurs, the sight information gathered from the eyes is received and converted to data, but it is not processed and thus the consciousness experience is absent. The information is still flowing through the sight-involved areas, but it is the awareness content to suffer from the processing loss. Blindsight has comprehensibly been subjected to controversy because of the difficulties met when trying to prove its existence and symptoms. Consciousness, in this case, cannot be properly measured with physical means as it would at most based on a patient's report [1] [14] [15] [16].

Materials

Chapter 4
EEG

4.1 Brain Signal

Brain researches differ first of all according to the scale of the explored area, and then depending on the required technology. Three main scales can be identified, respectively from the smallest to the largest:

- **Microscale**: analyzes single neurons. In this scale, direct measurements of intracellular voltage are carried out. It is a powerful tool, but tedious and invasive. It is limited to a few neurons at a time, using patch clamp or sharp microelectrodes. The electrodes are placed directly in contact with the brain, in contact with the analyzed neurons whose transmembrane potential is measured;

- **Mesoscale**: studies populations of neurons in a simultaneous and long-term recording. Microelectrode arrays (MEAs) and polythrodes are therefore required, which acquire signal directly from the inside of the brain, even during surgical interventions. This method of acquisition is as invasive as the use of single microelectrodes, but their usage is limited to the cerebral cortex in a way to reduce possible damage. The main acquisitions of this tool are Local Field Potentials (LFPs) and Extracellular Action Potentials (EAPs);

- **Macroscale**: analyzes large areas of the brain. Bioelectromagnetic signals generated by neurons in the cerebral cortex can be registered through two main techniques, which can be coupled and integrated with each other or with other tools [17]:

    - **Electroencephalography** (EEG) which measures from the scalp spontaneous or stimulated evoked variations of the electric fields generated by groups of pyramidal, with low spatial resolution (around centimeter range). The bioelectrical signal is acquired in a non-invasive way, through the activity of millions of neurons but the recorded signal corresponds to the signal produced by neurons near the surface of the brain, while the deep layers are not observable. Several physiological and environmental artifacts can affect the recording, including physiological one such as

eye-blink and muscle movements, and environmental ones such as cable motion and noise;

- **Magnetoencephalography** (MEG) which registers variations of the magnetic field due to variations of the electric fields generated by the same neurons [18];

- **Electrocorticography** (ECoG) or Intracranial EEG (iEEG) which is performed not on the scalp but by placing the electrodes directly on the surface of the brain, extremely invasive as it requires a craniotomy to place the electrode grid. This way artifact corruption in reduced or even completely removed.

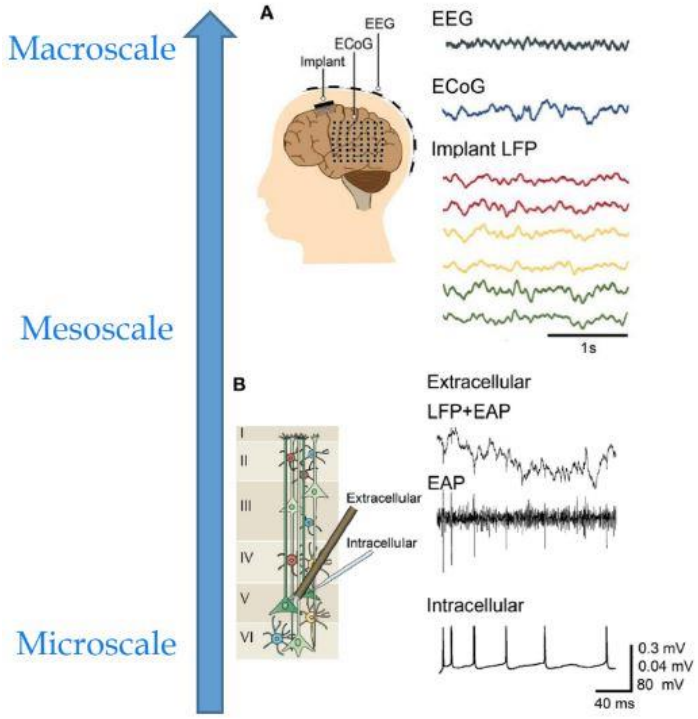


***Figure 9**. Representation of brain studies on different scales: Microscale for intracellular signals of single neurons, Mesoscale for extracellular signals of neuron populations and Macroscale for EEG and ECoG from large areas of the brain [20].*

4.2 Introduction to EEG

Electroencephalography (EEG) is a non-invasive brain's electric activity measurement. It is accomplished by placing a certain number of electrodes on the scalp of a subject to register the potential related to a passing current which flows through neurons

It is an electrical signal which can be registered from the scalp, and it is due to the action of neurons constituting the cortex. The activity of the neurons is not synchronized, aside from specific pathological cases or when experimentally induced. The registered signal, thus, results as noise, and it is quite difficult to recognize standard waveforms. Unlike ECG signal which is almost deterministic and periodic, EEG is a random process, and it can only be described in statistical terms. Its first applications go back to the ending of 1800 and the beginning of 1900. It was considered the sole way

to observe the brain's activity until the 70s. During the period going from 1920 to 1960 EEG evolves and becomes more modern, facing different employments in clinical diagnostics and neurorehabilitation treatments. Thanks to the innovations in signal processing that have followed one another over the years, it has been used both as an experimental tool and as a neuroimaging method. In the 70s, though, CT and MRI show up, enabling to get anatomical images of the brain in a precise and detailed manner. Since most pathologies are associated to anatomical anomalies of the brain, the diseases' management was then entrusted to imaging analysis and, as a consequence, EEG started to lose importance from the clinical point of view. A lesion in the brain can be localized with a spacial resolution inferior to the millimeter using imaging diagnostic tools, while the source of an EEG signal could never be localized which such precision. The main contribution of EEG is given from its temporal resolution: CT and MRI have low time resolution, while EEG is insuperable in localizing a phenomenon in time. A variation in the electrical signal inside the skull can be recorded and read instantly all over the scalp. In the 90s it was decided to match the spatial information gained from bioimages and the temporal ones derived from EEG, but the results never reached all the expectations. EEG, in the end, became the irreplaceable means of two main clinical applications: study of epilepsies and ascertainment of cerebral death. Other applications involve the study of evoked potentials through the application of external stimuli and sleep-cycle analysis. Besides, EEG suffers from several artifacts, starting from movement artifact, but in the years the analysis has improved in means capable of removing or reducing them. The electrodes placement for signal acquiring has been standardized, mainly regarding their position, more than their number. In modern EEG applications, though, a large number of electrodes can be employed to get high-resolution signal for research applications. The latest emerging application of EEG concerns neuroprothesis which allow disabled people to get interfaced to a machine only using their EEG signal [21] [22].

4.3 History of the discovery of EEG

Richard Caton

It was Richard Caton in 1875 to acquire for the first time an electrical brain signal from the surface of an exposed brain of animals. He used a reflective galvanometer with some modifications to create a sort of primitive amplification. This way, weak currents of micro-Amperes were made measurable. In 1887 Caton detected the first variations of electrical brain activity due to an optical stimulus. He also noted that stimulating one eye, it was the opposing side of the brain to activate [23] [24] [26].

Adolph Beck

Adolph Beck in 1890 was able to recreate Caton's work, discovering, in addition, that visual and auditive stimuli were able to interrupt at a single point the slow patterns of wave in the whole brain, anticipating the notion of a reticular activating system, which was discovered only fifty years later. He localized sensory stimuli evoked brain potentials placing electrodes directly on the brain surface and he was able to observe brain waves desynchronization and fluctuations in brain activity which stopped as the sensory stimulation ceased. Beck can be viewed together with Berger as one of the founders of electroencephalography [25].

Waldimir Neminski

Researcher Wladimir Neminski in 1913 gained the first spontaneous electrical brain registration, identifying two main rhythm patterns of the electrical activity. These were at first named as first and second order waves, then changed to A-waves and B-waves, until they became the modern alpha and beta-waves [25].

Hans Berger

In 1924 the first human electroencephalogram was recorded by Dr. Hans Berger. He is the one who coined the term "electroencephalogram". He based his work on Caton's contribution and his studies involved both normal and abnormal EEG phenomena, associated with attention, injuries and signal alterations. It was only in the 1934 that his reports started to be considered as reliable. Berger was the first to publish EEGs coming from humans. His studies revolved also around the cerebral capabilities of intellect and damage perseveration after injuries in prefrontal cortex and frontal lobes. He pointed out that the best recordings came from occipital electrodes with frontal reference. In addition, he assumed that blood circulation and the skin could affect the EEG registration, so he started recording with simultaneous ECG and blood pressure measurements, and just below the skin. He is the one who characterized the electrical wave pattern, alpha and beta waves included. Alpha waves were named this way because they were the first ones to be isolated in human EEG. His recordings were made on moving photographic paper with waves at 10 cycles per second. These waves were found to diminish during sleep and anaesthesia. Alpha waves were proven to have decreased frequency in injured subjects and large amplitude in epileptic patients. He was able to find correlations between EEG waves and other diseases like Alzheimer's and multiple sclerosis, but not other illnesses. Beta waves were instead reported as lower in amplitude and related to concentration and startle reaction.

Despite Berger's work was at first ignored by the scientific community, in 1934 Cambridge Physiological Laboratory researchers Adrian and Matthews confirmed his studies. This allowed Berger to continue his studies on cerebral activity, focusing on EEG phenomena such as epilepsy. At the same time, his studies allowed following neuroscientists to proceed using his technique for clinical applications and neurophysiological research [23] [26].

Herbert Jasper

Herbert Jasper's studies are recognized even now as the basis for brain science for his contribution in brain activity research related to consciousness and learning. He was a pioneer in EEG pathophysiogenetic study of epilepsy and he was able to record the activity of single neurons with microelectrodes, analyzing the neurochemical mechanisms at the base. He founded the EEG Journal and was member and president of the American EEG Society. His main accomplishments are related to EEG recording directly from the cerebral cortex and his "reduced" montage used for quick EEG exams like those needed in ER [27].

4.4 Biopotentials

4.4.1 Neurons

Neurons are the basic unit of the brain. There are supposed to be around 100-150 billions of neurons in a human brain, each with 10^3-10^4 connections. Inside the brain there are also glial cells to support and protect the neurons. The main role of a neuron is to transmit and receive nervous impulses. Bioelectrical signals can be sent to other neurons or specific organs.

A neuron is composed of three main parts:

- **Cell body**: with a nucleus, organelles and cytoplasm. DNA and information for cell's growth and metabolism is contained in the nucleus;
- **Dendrites**: they are the main link between neurons, as they receive the electrical and chemical impulses through their ramifications from other neurons or sensory organs and integrate information in the axon hillock;
- **Axon**: they branch from the cell body off to other neurons sending stimuli. The stimulus is an action potential, that is a brief electrical signal which travels at great speed [28].

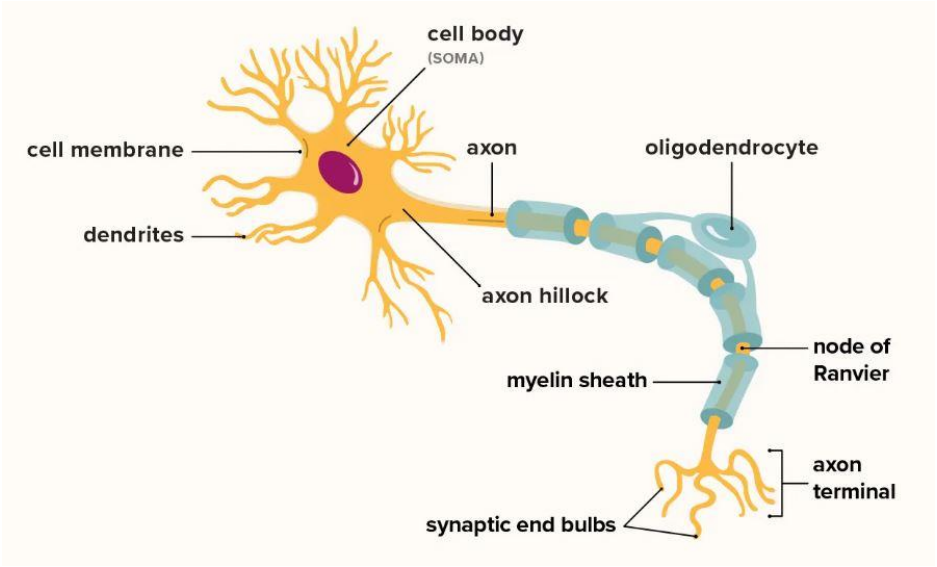


Figure 10. Structure of a Neuron [29].

The cell membrane separates the inside from the outside, controlling the environmental system.

Synapses, on the other hand, represent the anatomical structure where the communication between neurons happens. Information is sent through neurons as a biosignal right by synapses.

More than 10^4 specific types of neurons can be counted in the human brain, all of different sizes and shapes. The main classification can be done dividing:

- *Sensory neurons*: they transmit information from receptors from the skin or the retina to the CNS. They have long dendrites and short axons and are afferent neurons;

- *Motor neurons*: they transmit information from CNS to effectors like muscles or glands. They have short dendrites and long axons and are efferent neurons;

- *Interneurons*: they connect motor and sensory neurons within the various regions of the CNS. They have short dendrites and axons of which can be long or short. They integrate activity between afferent and efferent neurons.

The neurons can be easily distinguished in the spinal cord, but their distinction is way more complex in the brain [28].

4.4.2 Synapses

When EEG signal is recorded, the main contribution in the transmission cell to cell of the bioelectrical activity is given by the synapses. They represent the joint between two neurons which communicate with an action potential firing from one neuron, the presynaptic, to the receiving one, the postsynaptic. There have been controversies about whether the signal transmission of the synapses was chemical or electrical because it was thought that the transmission was due to a flow of ions directly from one neuron to the other, but at the same time it could just be due to the release of a chemical from one neuron which caused a response in another one. Nowadays, both solutions are accepted as there can be chemical synapses and electrical synapses. The chemical transmission, though, might be more common yet complicated.

In the **chemical transmission** a neurotransmitter is involved, which is a chemical messenger carrying information from the sending neuron to the receiving one. Synapses form between the axon terminals of the sending neuron and the dendrites of the receiving neuron. A single presynaptic neuron can make a synapse on many postsynaptic neurons because the axon can branch multiple times. The same can happen to the receiver that can be connected to many sending neurons. In the axon terminal there are synaptic vesicles bound to the membrane and filled with neurotransmitter. A small gap is present in between of the axon terminal of the sending neuron and the membrane of the postsynaptic one, called synaptic cleft. The impulse of an action potential activates the calcium channels in the membrane. Ions on the outside flow inside the cell, making the vesicles to fuse with the membrane of the axon terminal, releasing the neurotransmitter in the synaptic cleft. At this point the neurotransmitter diffuses through the cleft, connecting to the receptors on the postsynaptic neuron. When these receptors activate, ion channels on the cell membrane close or open, depending on whether it depolarizes or hyperpolarizes, making the inside of the cell respectively more positive or negative, and this is decided by the involved ion [30]]31].

The neurotransmitters with major effects are:

- Glutamate: major excitatory transmitter
- GABA (Gamma-amino-butyric acid): major inhibitory transmitter
- Acetylcholine: activates muscles
- Dopamine: promotes motor activity, motivated behaviors, endocrine control
- Norepinephrine: fight-or-flight response

- Serotonin: controls mood, sleep and hunger.

As the receptor activates, the membrane potential undergoes a change, and depending on the case, it may make the cell more or less likely to fire the action potential. The kind of neurotransmitter decides whether the postsynaptic potential is excitatory (**EPSP**) or inhibitory (**IPSP**). The EPSP is depolarizing and brings the membrane potential close to the firing threshold for an action potential, and it can sum to other EPSP in order to reach the threshold value. The IPSP works in an opposite way, lowering the potential below the threshold, cancelling the effects of EPSPs.

The effects of EPSPs and IPSPs is progressively added and integrated: integration happening in different locations, at the same time, is called spatial summation, while the integration occurring in the same place in different times is known as temporal summation.

Once the signal is sent, it ends only when the synaptic cleft is cleared of neurotransmitter. In fact, it can be treated by an enzyme, it can go back to the presynaptic neuron or just freely diffuse.

Electrical synapses, differently from the chemical ones, connect presynaptic and postsynaptic neurons with a physical link. The connection is known as gap junction and it is a channel which allows ions to flow from one neuron to the other. The transmission of the signal is quicker in respect to the chemical synapses'. The fast information allows synchronized activity of groups of cells but lacks signal modulation. In addition, some synapses can be both electrical and chemical, and the response in these happens before in the electrical manner and then in the chemical form [32].

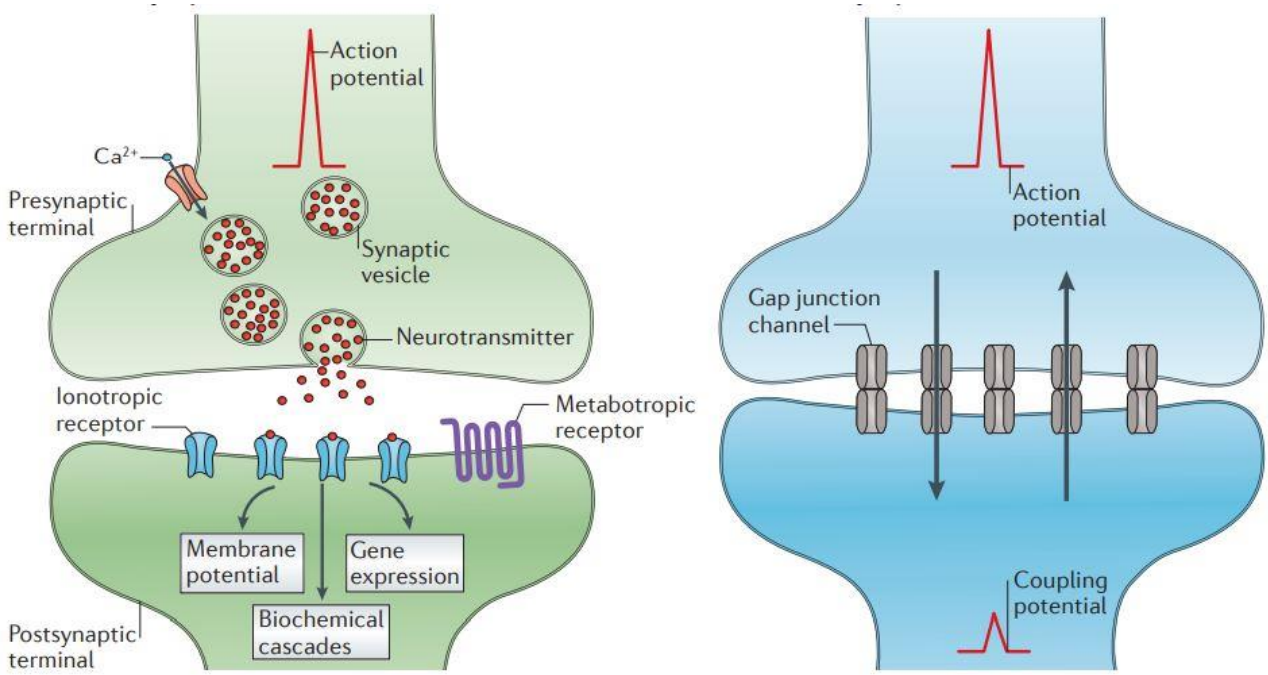


***Figure 11**. Chemical Synapse transmission (left) and Electrical Synapse transmission(right). The main differences are highlighted: in chemical synapses, presynaptic and postsynaptic neurons are separated by the synaptic cleft and neurotransmitters diffuse to activate specific receptors that regulate the postsynaptic events; electrical synapses present a physical link with channels that allow ionic currents to flow freely [32].*

4.4.3 Biopotential Acquisition

The bioelectrical phenomena are analyzed according to the signals deriving from axons, dendrites, cell body, and the EPSP and IPSP. Axons and dendrites seem to not contribute to the cortical waves of the surface as they put resistance to the flow of ionic current lowering its amplitude, and the electrodes on the scalp capture just ionic current. In addition, the cell body develops an action potential of a way shorter duration, around 1 ms, compared to the cortical waves, so they seem neither to contribute to the surface cortical wave because to get a recordable activity a major neuron synchronization would be needed.

On the other hand, EPSPs and IPSPs produce various slower waveforms of duration and amplitude comparable to the cortical electrical signal, which can be acquired even without a strict synchronization of the neurons. This shows a strict correlation between the EEG rhythm and postsynaptic potentials making the latter the main contributors for EEG and MEG acquisition [33].

4.5 Electrodes

4.5.1 Electrode types

EEG is recorded through electrodes which are placed on the scalp of a patients to measure and record a bioelectric signal in the form of ionic charge transformed into electric current. The electrode is basically a transducer made of a conductor material placed in an ionic aqueous solution represented by the skin or a specific gel. There are different materials for the different types of electrodes [1]. The main categories are:

- **Metal plate electrodes**. They are the most popular type of electrode. They once required low contact impedance, so they needed large with an area of around 10 cm^2 or more. Nowadays it is possible to get higher contact impedances, so the size of the electrodes has been able to be reduced. They are typically used with a disc form, with a diameter of 3-4 millimeters, but it can get up to 1 centimeter. They are made of stainless steel, because of their good properties in the scope of biopotential acquisition. The electrodes usually are covered in other materials, such as platinum, gold, or silver chloride. The covering allows to get a more suitable contact surface in order to reduce artifacts and noise due to movements, maintaining the costs low. In fact, an electrode covered in gold and one made of pure gold from the start are equivalent from the contact point of view. Some electrodes have no coating but are coupled with a spongy net soaked in physiological solution. This enhances the electrical performances of the electrode. Such stratagem allows the electrode to be used many times even on different subjects.
  EEG electrodes can be placed manually and one at a time, or my simply wearing a cap integrated with an electrode grid which contains all the electrodes in position. A grid is also adopted for iEEG recording, placed under the dura or between a sulcus [22].

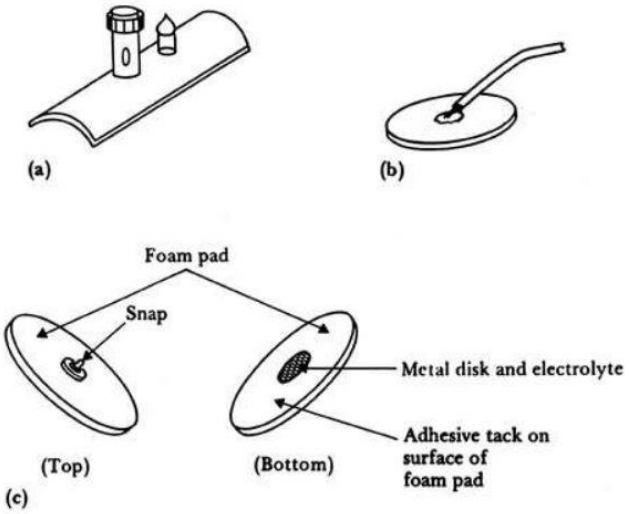


Figure 12. Metal-plate electrodes for generic Biopotential acquisition on body-surface. a) On limbs; b) Applied with surgical tape; c) With disposable adhesive foam [33].

- **Floating electrodes**. They are discoidal metallic electrodes in no direct contact with the skin of the patient. In fact, they are placed at a certain distance using a case which is then filled with an electrolytic gel acting as an intermediate layer between skin and electrode. These electrodes are extremely useful for their properties and for their ability to significantly reduce movement artifacts [22] [34].

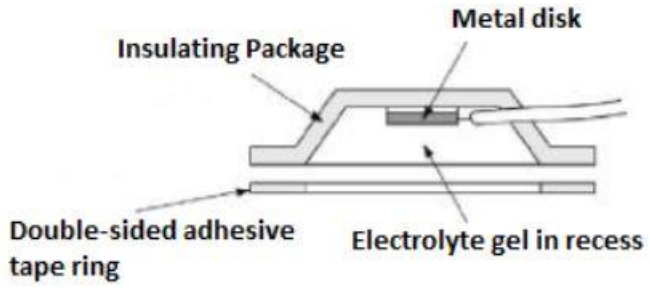


Figure 13. Floating electrode, separated with a case from the skin [34].

- **Flexible electrodes**. Used when an electrode is placed on a specific area with a tight bend radius, useful with small subjects such as newborns. They can be made of rubber made conductive through a dispersion of graphite and silver salts.

- **Invasive electrodes**. They penetrate or even pierce the human body and human brain in the form of needles. They can be arranged in an array, both mono- or bi-dimensional. They are utilized in neuroengineering to acquire the potential directly from the inside of the brain. They are narrow, 1-1.5 millimeters wide, made of different materials such as silicon with the electrode place right on the top of the needle. Microelectrodes can differ depending on the

material, which can be a metal or a glass, and the processing method to create them, such as electro-erosion or heating [22] [35].

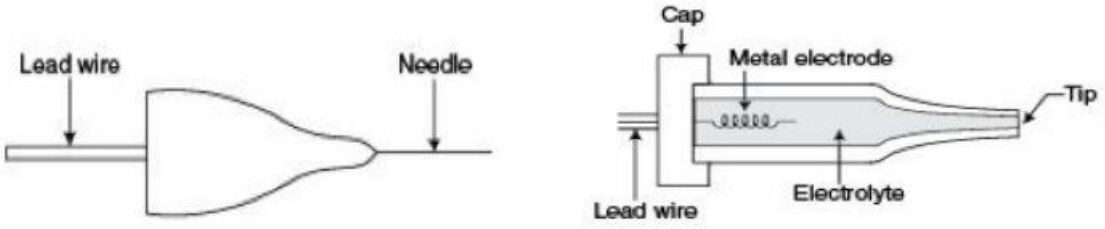


Figure 14. *On the left is a simple representation of a Needle Electrode for EMG or EEG uses. On the right a more Micropipette Electrode for intracellular potentials [22] [35].*

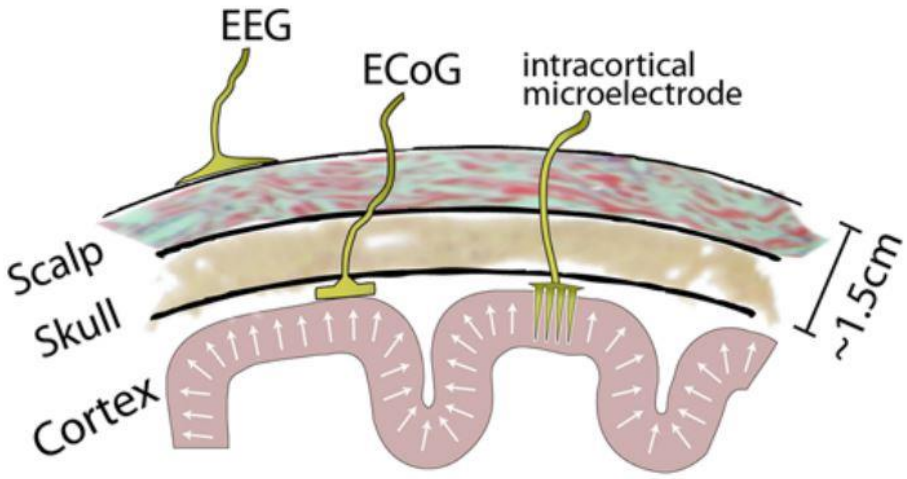


Figure 1. *Difference in the position and depth of EEG, ECoG and Intracortical electrodes [36].*

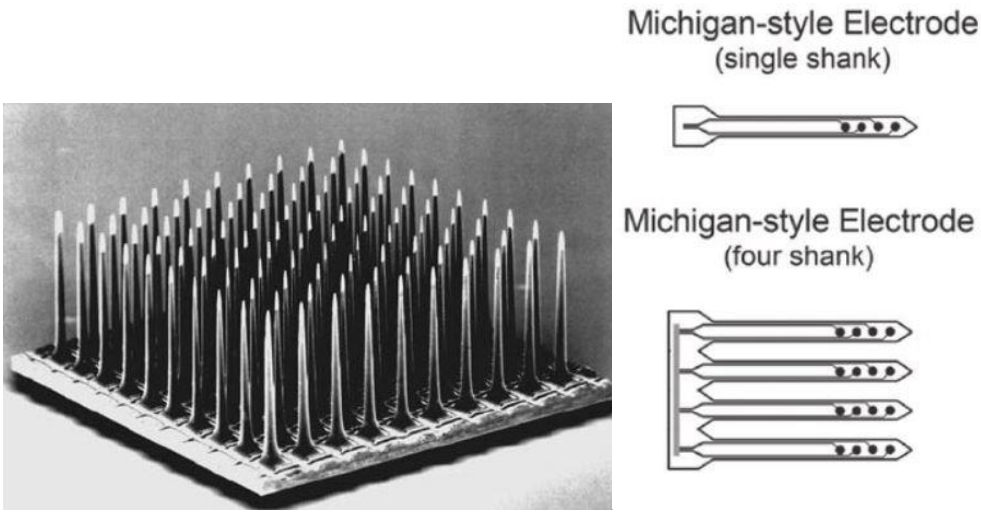


Figure 16. *Microelectrodes used in Neuroengineering applications:*
Utah MicroElectrode Array (left) is composed of an electrode matrix, each separated and isolated from the others [37];
Michigan MicroElectrode Array (right) is a single array of electrodes placed on a single or multiple shanks [38].

4.5.2 Electrodes placement

Placing the electrodes in the correct point is important because in every zone the signal can be different and represent a phenomenon or another. To be sure that the correct electrode placement is set, some standards were designed by the International Federation of Societies for Electroencephalography to get universal recordings. These standards give indications about some of the main landmarks all over the skull. This way, with some iterations, all the electrodes can be correctly placed.

It is important to note that the standards do not impose to use a certain number or electrodes. As a matter of fact, not all electrodes could be placeable, or would be needed. So, the standards limit to tell which the possible points of the skull are where an electrode might be placed. In addition, unlike for example ECG signal, EEG requires a large number of electrodes, ranging from 8 even to around 80, although the usual number is set to 21.

The most important is the **10-20 standard**, but there are others such as 10-10 standard. The 10-20 standard starts by giving four skull landmarks:

- *Nasion*: the pit above the root of the nose;
- *Inion*: collocated posterior to the skull, on a slight protrusion just before the termination of the occipital bone;
- *Left* and *Right Pre-auricular points*: under and just anterior to the auricle pavilion, located on both sides of the head.

These are the starting points from where all the electrodes' positions are obtained, in particular:

- **F** electrodes are placed over the frontal lobe
- **P** over the parietal lobe
- **O** over the occipital lobe
- **T** over the temporal lobe
- **C** represent the central electrodes
- **Fp** above the eyes
- **A** on the ear lobe.

Looking frontally from above, the vertical central electrodes are marked with a letter z. while the left side of the skull is marked with odd numbers associated with the mentioned letters, and the right side with even numbers. Cz electrode is the first positioned, measuring the 50% of the distance between Nasion and Inion, and from one pre-auricular point to the other. Starting from Nasion to Ision, counting respectively the distance of 10%, 20%, 20%, 20%, 20%, 10%, electrodes Fpz, Fz, Cz, Pz and Oz are marked. Measuring in the same way from Fpz to Oz from both sides allows to obtain the lateral electrodes' positions, while the remaining numbered ones derive from intersection of the previous positions. Intermediate and supplementary electrodes can be added by covering the midpoints between the others previously marked. The entire scalp results in a grid defined by the chosen number of electrodes and might vary. Wearable caps exist which contain all the needed electrodes, allowing an easy electrodes' placing [41] [43].

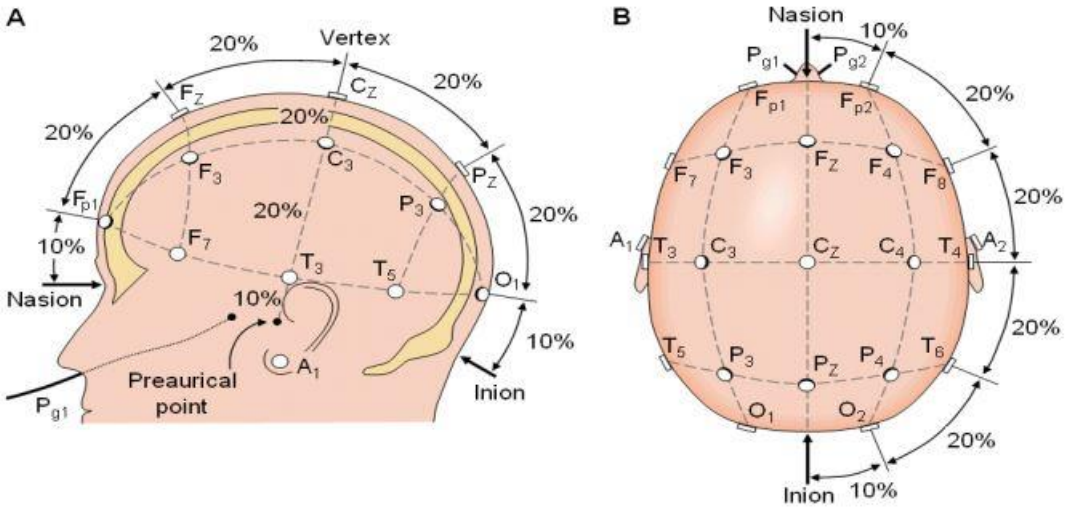


Figure 17. *A) Lateral and B) Dorsal view of the scalp's mapping for EEG electrodes placing [42].*

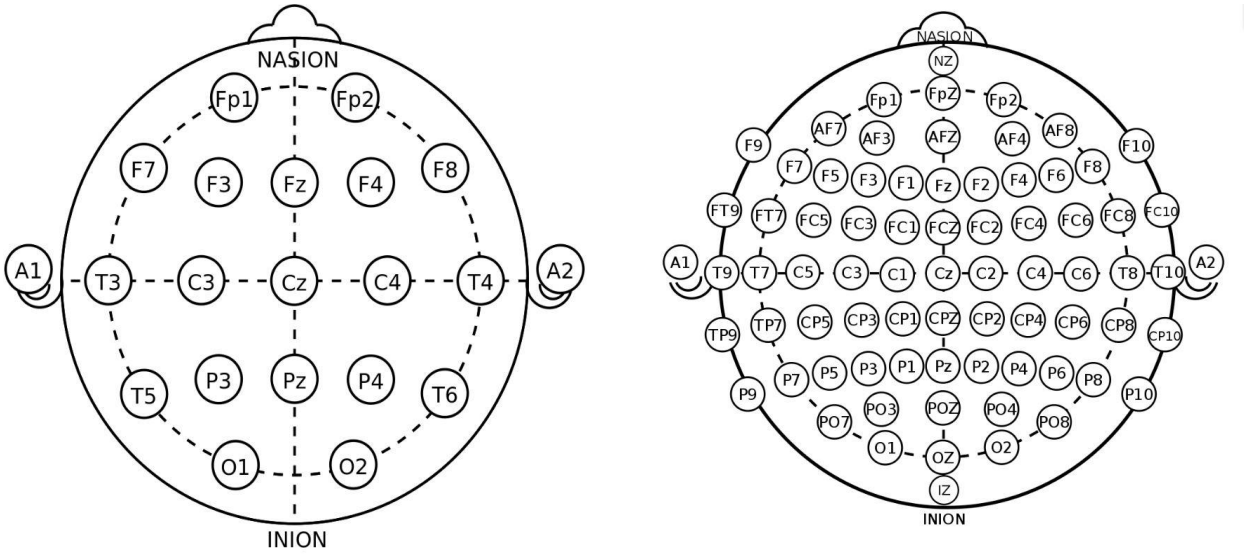


Figure 18*. International 10-20 Electrode Placement System: On the left the main electrodes used by the system; on the right the electrode map completed with all the intermediate electrodes deriving from the main ones [43].*

4.6 EEG Montage

Once the electrodes are placed, how to connect them to the amplifier needs to be decided. Two types of montages are available:

- **Differential montage**: in this case, the electrodes are taken to a differential amplifier. The signal results as the voltage difference between two electrodes. N electrodes lead to N-1 difference signals. Whenever a signal source should be located in the middle between two electrodes, their potential would be the same, and thus their difference equal to zero. On the other hand, a larger difference would be registered with a single electrode near the source and the other farther.

- **Monopolar montage**: in this case, there is a common reference which acts as a second input associated with the EEG channel. Since the reference is the same for all channels, a source near an electrode will produce a wider signal compared to other located farther. As a reference, usually electrodes A1 and A2 are selected, just under the auricle pavilion, but other options are usable, such as the mastoid bone behind the ear or even an electrode placed under the chin. Sometimes, even a non-cephalic reference is adopted, using the weighted average of the potentials measured on the neck between the first two cervical vertebrae [1] [22].

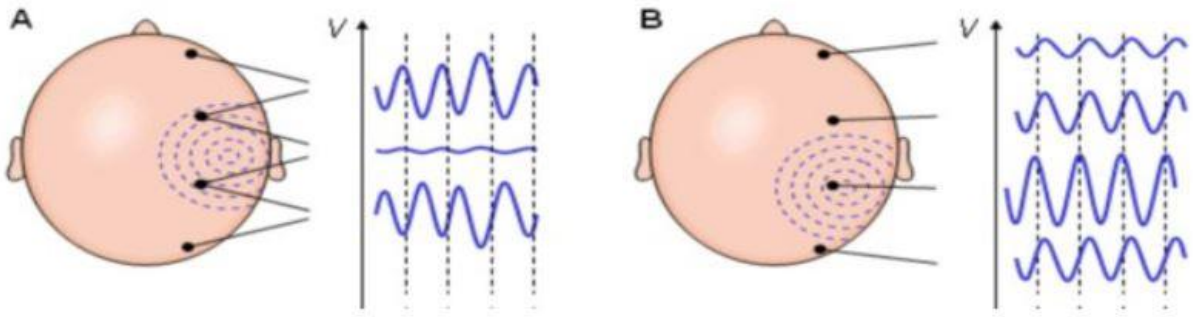


Figure 19. A) Bipolar and B) Unipolar EEG acquisitions showing the differences in the recording in relation to the source [42].

4.7 EEG characteristics

Once the EEG is recorded, there are many parameters and characteristics that can be analyzed to define it:

- **Amplitude**: ranges between 10 and 500 microVolts, distinguished between low (<30 uV), medium (30-70 uV) and high (>70 uV). The measured amplitude strongly depends on the adopted montage, as in differential montage the true amplitude of a signal is not represented by the recordings between two electrodes.

- **Morphology**: can be polymorphic and monomorphic, representing the repetitiveness of a determined frequency in a signal.
    - *Polimorphism* means a succession of potentials belonging to the same frequency band, with a non-regular amplitude, non-periodic and differente from one component to another;
    - *Monomorphism*, instead, refers to a regular succession of potentials having all the same frequency and amplitude.

- **Topography**: defines the cerebral area where an electrical event manifests. It is identified referring to the classical anatomical distinction among the cerebral hemispheres and lobes.

- **Transient** and **burst**: they represent sudden activity clearly recognizable from an EEG. In particular, a transient is identified with a single wave, like a spike discharge, while a burst with a group of waves. They are crucial for seizure analysis.

- **Symmetry/Asymmetry**: symmetrical signals present same characteristics of frequency, amplitude and duration on both hemispheres, while asymmetrical ones occur only on one of the hemispheres, or, when on both, they present different characteristics.

- **Synchrony/Asynchrony**: based on the moment of appearance of events on both hemispheres.

- **Reactivity**: it is an indicator of a reaction of the patient to a particular stimulus.

- **Frequency**: EEG ranges from frequencies of 0.3 Hz to about 80 Hz, but it is usually classified in different frequency bands, even if they are not standardized and not constraining:

    - **Delta waves** (0.3 – 4 Hz, 20-200 uV), appearing in newborns and during the sleep cycle in adults. They indicate cerebral lesions and diseases when they appear in wake adults;

    - **Theta waves** (4 – 7.5 Hz, 5-100 uV), in childhood and drowsy adult subjects. Their presence in wake adults is abnormal and sign of some sort of pathology;

    - **Alpha waves** (8 – 13 Hz, 10-200 uV), in adults during wakefulness, present most of all in the occipital lobes of the brain. They present during physical relaxation and are best observed in closed-eyes patients. They get attenuated, instead, when visual stimuli and alertness occur;

    - **Beta waves**, divided in Beta1 (14 - 21 Hz, 1-20 uV) and Beta2 (21 – 40 Hz, 1-20 uV), present in the frontal lobes in healthy adults. They are associated with concentration and active thinking;

    - **Gamma waves** (>40 Hz, 1-20 uV), similar to the Beta waves, appearing in adults in an attention state [1] [22] [45].

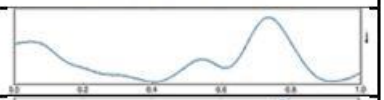
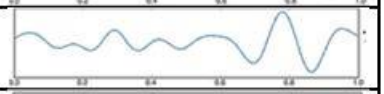
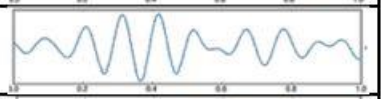
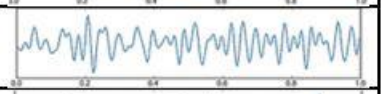
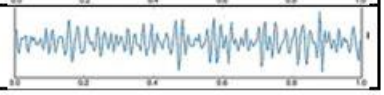
Waves	Frequency bands (Hz)	Behaviour Trait	Signal Waveform
Delta	0.3 – 4	Deep sleep	
Theta	4 – 8	Deep Meditation	
Alpha	8 – 13	Eyes closed, awake	
Beta	13 – 30	Eyes opened, thinking	
Gamma	30 and above	Unifying consciousness	

Table 1. *EEG bands division according to frequency range and condition of presence [44].*

4.8 EEG as a random process

An EEG signal track acquired on the scalp of a resting patient can be viewed as a **random process** because it is generated as a result of the casual superimposition of the electromagnetic fields produced by the post-synaptic potentials of the activated neurons in the acquisition volume. The EEG track is thus non-stationary and non-linear. Also symptoms and pathological electrical events appear in the form of random processes, and in order to extract useful information for diagnosis and classification, different processing techniques are often required. As a first step, the effect of noise and artifacts should be removed, then different EEG events can be studied in the time and/or frequency domain, especially isolating the different frequency bands [44].

4.9 EEG states analysis

Various events have effects on the EEG signal. The main ones are sleep, epilepsy, anaesthesia, meditation, and various artifacts.

Epilepsy

One of the neuronal pathologies that can be diagnosed using EEG is epilepsy. It is a disease lead by abnormal neuronal activity. In detail, spikes or sharp waves may appear, and they can occur occasionally or many times a day, suggesting serious diseases. Even the affected area may change or be specific in some brain areas, indicating a potential seizure activity. EEG becomes crucial for distinguishing epileptic seizures from other kind of disorders like a syncope, to differentiate

encephalopathy from psychiatric syndromes, to determine the right medication and localize the region where the epilepsy originates [46].

Sleep

EEG represents the most used tool for sleep analysis. This kind of research allows to differentiate between five sleep stages: Stage 1 is a light sleep, where muscle activity slows down with twitches; Stage 2 is the longest and is the one where breathing and heart rate slow down, decreasing the body temperature; Stage 3 is where deep sleep begins and the delta waves get slower; Stage 4 represents very deep sleep, with rhythmic breathing and limited muscle activity. Delta waves are here produced by the brain; in Stage 5, instead, there's a rapid eye movement and the waves accelerate. It is the part where dreaming occurs, muscles relax, breathing gets fast and heart rate increases [47].

Mu waves

Mu waves are peculiar waves which appear at 7.5 – 12.5 Hz. They are only present in the motor cortex and are suppressed by voluntary movement or intentional movement. Their main application regards virtual controls, like cursors, for Brain Computer Interfaces (BCI). Their frequency range overlaps with the alpha waves' one, but the main difference relies in the maintenance of the mu waves after eye opening [48].

Mirror neurons

Mirror neurons were discovered in the pre-motor cortex of monkeys. They are present in humans too, but they work differently. They are neurons that shoot both when a motor action is accomplished, and when a motor action is seen accomplished by others. The similarities of the shoots depend on the task and their activation relies not much on the action, but on the moment during which the purpose of an action is intuited.

A deficit in imitation, empathy and pragmatic language is typical of autism spectrum disorders (ASD). Researches have tried to prove how mirror neurons dysfunctions might be correlated to ASD. Mu waves play a central role in the case, as they are only present in the sensorimotor cortex, and they would reflect the activity of mirror neurons. Mu waves are suppressed in ASD individuals, comparing recording during accomplishment and observation of actions [49] [50].

Evoked potentials

Evoked potentials derive from an external stimulus used to verify the integrity of the nerve conduction pathways. A patient might suffer from muscle weakness and the evoked potential could tell if the problem comes from the nervous system or the muscle itself. In addition, nervous systems' problems could derive from the different motoneurons at different quote of the spinal cord. Evoked potentials are classified according to their polarity (positive or negative) and their latency in respect to the stimulus, measures in milliseconds.

They are divided according to the type of stimulus:

- *Somatosensorial evoked potential*, stimulating the tibial or median nerve;
- *Visual evoked potential*, stimulating the visive cortex through the retina;
- *Auditive evoked potential*, where the stimulus is in the form of a click to one ear and white noise to the other [22].

Holter EEG

Holter EEG is an EEG registration tool which allows to acquire a signal for at maximum 1-2 days from 4-8 channels. The montage is bothersome as it requires to wear the cap all the time and, thus, they are used as an ultimate resource, also because it is affected from many movement artifacts, and it is difficult to evaluate.

Human-Machine Interface

They represent one of the most current study subject, used to acquire a signal from a wheel-chaired or severely disabled people, with the use of a simplified Holter, in order to communicate through the signal deriving from a visual stimulus which could move a cursor on a machine [17] [22].

4.10 EEG artifacts

EEG signal is affected by several major artifacts, especially when compared to other biopotential acquisitions. It is not simple to distinguish them from the signal because of their comparable shape, frequencies and amplitudes. Some of them might be localized to certain channels, due to certain predictable causes or periodically identifiable. The main artifact which can be found are:

- **Eyeblink artifact**. It is due to the movement of opening or closing eyelid. It is easily recognized because of the involved channels and can be removed using the adequate filters. It appears, in fact, in the delta frequency band, so it must be decided whether it should be removed or not, because removing the artifact also removes the delta band which could be interesting to analyze.

- **Cardiac artifact**. It manifests simultaneously in all channels, in a certain interval, typically of 1 second. It can't be removed because it would exclude important components of the EEG signal.

- **Ballistic** or **Pulse artifact**. It is generated by the movement of an arteriole on the scalp. Recognizable due to the periodicity and the appearance on 2 opposed channels in differential montage. It might be avoided simply by repositioning, if possible, the affected electrodes, but it can't be filtered.

- **Artifact at the network frequency**. It is localized to certain channels, so it is not due to Common Mode Rejection Ratio (CMRR), nor the impedance level. It is, instead, an artifact due to a couple of unbalanced electrodes which, if not well positioned in a montage phase, shows the common mode component at 50 Hz in Europe and 60 Hz in the United States.

Other artifact causes can be related to: the eye movement (different from eyelid movements), both vertically and horizontally, as they create different effects on different channels; facial muscle activation, electrode pop due to rapid charge distribution under the electrode, and breach due to flaws in the bone structure of the skull [51] [52] [53].

Chapter 5

Entropy

5.1 Introduction

Biomedical data such as EEG, ECG and EMG are characterized by complex behavior and non-linear properties. To study said signals, the stationary condition must be assumed, even if it is not usually satisfied. This is overcome by dividing the starting signal in brief intervals during which the system might be assumed to be in a quasi-stationary state. In addition, biomedical data can be extremely noisy. **Approximate Entropy** (ApEn) was introduced as an index capable to describe the complexity of a signal even from noisy and short epochs. Its parameters define the reliability of the estimations, and they are chosen between ranges found in literature from previous experiments. The main ones are:

- the **embedded dimension** (m), representing the length of the data runs
- the **epoch duration** (T)
- the **filtering level** (r), which is a threshold for a recurrence to be found
- the **sampling frequency** (fs), as it was proven to give better approximate estimations when selected at the Nyquist limit.

Comparisons among researches are difficult to make because of the wide range of possible adopted values for all the parameters. Reproducibility is a common problem because successful results sometimes might be obtained after tuning certain parameters adapting it to the wanted outcome. In addition, even if a work should show a large ApEn results, it would not obligatorily imply a higher complexity. This leads to contradicting literary results, other than the possibility that a given effect might result by just blindly changing parameters. The impact of the variation of one of these is not predictable and the optimal choice often relies on an extremely accurate search of a trial-and-error approach. To avoid all said parameters setting problems, some criteria are established on the basis of repeatability, reproducibility and possibility of comparison among works.

Complexity was computed and compared in a wide range of clinical conditions. ApEn resulted to increase during epileptic seizures, allowing to localize and sometimes even predict the event. A lower complexity was, instead, proven to appear for all of the following cases:

- In schizophrenic people;
- In Alzheimer patients;

- In Patients with attention disorder or hyperactivity;
- While applying specific stressors:
- With anaesthetic induction;
- In vegetative or minimally conscious patients.

ApEn was indeed employed to differentiate different states of consciousness during sleep. The wakefulness level was registered focusing of the analysis of alpha waves, allowing to distinguish five or even six different consciousness stages [54].

5.2 Approximate Entropy

Given a time series, Approximate Entropy reveals its fluctuations' predictability or unpredictability, and its complexity. It searches for repetitive patterns that, if present, make the signal less complex and more predictable. Thus, it allows to predict the behavior of a signal in the future, based on what has happened before.

Noting r as the tolerance and m as the embedded dimension, The ApEn represents the logarithmic probability for a time series to repeat itself within r, for both m and m+1 points.

The time series is embedded in the vector phase space:

$$X(i) = [x(i) \quad x(i-1) \quad \dots \quad x(i-m+1)]$$

x(i) represents sample number i of the series, while **m** is the length of the run. Considering **r** as the tolerance, the probability that **X(i)** is similar to other vectors within r is named correlation integral.

$$C_i^m(r) = \frac{N_i^r}{N-m+1}, \qquad i = 1, \dots, N-m+1$$

Here $\mathbf{N^r_i}$ represents how many vectors have distance lower than r from the embedded vector X(i), while N stands for the number of samples. L-infinite norm is used for the distance definition. Since the X(n) vectors draw a trajectory in the phase space, the correlation integral $\mathbf{C^m_i(r)}$ as a definition divides the recurrences $\mathbf{N^r_i}$ of point close to X(i) by the possible number of pairs.

The approximate entropy is given by the correlation integral obtained for two consecutive embedded dimensions:

$$ApEn(m, r, N) = \phi^m(r) - \phi^{m+1}(r)$$

Where:

$$\phi^m(r) = \frac{1}{N-m+1} \sum_{i=1}^{N-m+1} \ln C_i^m(r)$$

Lower embedded dimensions result into higher number of recurrencies because the number of elements in the vector increases going from m to m+1. Recurrences in m+1 are surely also found in m, hinting that ApEn takes on larger values when the trajectories diverge [54].

5.3 Approximate Entropy's problems

- Embedding dimension needs to be large enough to avoid false near neighbors. Low m values could lead to projections of the trajectory to a lower dimension. The false neighbors would affect the ApEn value, but they would be unrelated to the system complexity and, thus, misleading.

  The inventor of ApEn, Pincus, gave some advice for using the parameters within the right range:

  - **m** = 2 or 3
  - **epoch duration** >= 10^m or 20^m samples
  - **r** = 20% std of the signal.

  High values for the embedded dimension, though, lead to a decreased probability to find recurrences. ApEn is strongly affected by low recurrences numbers because the denominators of Φ^m and Φ^{m+1} could bring to negative ApEn values.

- ApEn is susceptible to the value of the **sampling frequency**. In fact, ApEn results lower if the signal gets over-sampled because a linearization of the signal occurs. More samples imply that each sample is closer to the nearest one, making it more easily predictable and less uncertain. In fact, the maximum value for ApEn is given by ln(N-m), which increases as does the sampling frequency and, therefore, the ApEn value compared to its maximum value reduces with oversampling.
  The suggested sampling frequency value is around the Nyquist limit, with the embedded dimension equal to the ratio (Sampling Frequency)/(Nyquist limit).

- **Rare events** are more informative because of the weight given by the logarithm in the formulation of ApEn. This might result unstable whenever short epochs are used, as in this case rare events are not enough represented.

- **Low frequency trends** affect the recurrences which could not be identified if translated around different mean values. High-pass filters should be employed to remove the trends whenever possible. This is the case of signals behaving in the same way at different scales,

with their frequency components independent one from the other. In reverse, it is not possible when state changes occur in the system, that is when in a non-stationary state.

- According to the ApEn formulation, close in time values are also considered neighbors, leading to singularities that can be removed. Self-recurrences for N-m-1 are always found, shifting the ApEn result to lower values. This problem is overcome by two means:

    - introducing a **Theiler window** that defines a certain time interval in which no recurrencies can be found;

    - introducing **Sample Entropy** (SampEn) as a conceptually different measure of complexity which avoids singularities by replacing the logarithm with a summation [54] [55].

5.4 Modified Approximate Entropy

ApEn might be computed in a **modified version**, overcoming some of its limits:

- When considering the time delay (τ) between points, a linear correlation with the sampling frequency can be adopted as such. Delays different from 1 compensate over-sampling, avoiding self-recurrencies in a specific time window.

- To avoid finding too few recurrences, the tolerance (r) can be set at a specific percentage of the recurring points found in m dimension, instead of depending to the std. In some cases, r was set depending on the maximal ApEn value, showing both promising results and not.

- In the two embedding dimensions, $[N-(m+1)\ \tau]$ represents the total number of embedded vectors.

- A High-pass filter is employed to remove low frequency trends. It has a cutoff frequency based on the inverse of the epoch's duration.

- A Theiler window is used to not consider as neighbors points closer that a delay. When a recurrence is found in m dimension but not in m+1, a single recurrence is added to the correlation integral, while point which have no recurrences in dimension m are directly not considered.

The modified formulation of ApEn does not guarantee better results compared to its original one, because more tests would be necessary on a wider range of conditions and data. In addition, under certain circumstances, some parameters' combinations could lead to unstable or contradicting results.

There are, though, interesting results that prove some of the main perks of using the Modified ApEn:

- Compensated over-sampling, as the complexity is provided unrelated to the sampling frequency or the data bandwidth;

- The embedding dimension does not need to stay low, as the modified ApEn returns acceptable values even if m increases, unlike the original ApEn which would drop the number or recurrences;

- The epoch's duration is a major problem for the original index, as it needs to stay low to maintain a quasi-stationary state, but, at the same, the epoch cannot be too brief as the ApEn would not find enough recurrencies. This does not occur with the Modified ApEn when the proper embedding dimension is chosen.

- Compared to SampEn, self-recurrences are not required, so the local behavior of the signal does not affect the results. In fact, short time signal and rare events get to be represented as they should [54] [55].

5.5 Approximate and Sample Entropy in comparison

(On Quantization Errors in Approximate and Sample Entropy Dragana Baji´c 1,* and Nina Japundži´c-Žigon 2)

Both ApEn and SampEn formulations are based on similar template vectors and their matches. In ApEn there is also **self-matching**, which is the comparison of the template to itself. In SampEn matches can be absent, coming with the name of **zero-match**. In addition, with SampEn self-matches and the last comparison (N - m + 1) are not included.

Matching Probability is the probability for a template to find its match. It assumes two different, yet similar, formulations for ApEn and SampEn:

$$\hat{p}_{Ai}^{(m)}(r) = \frac{C_i^{(m)}(r)}{N-m+1}, \;\; i = 1, \cdots, N-m+1, \;\; \text{for } ApEn;$$

$$\hat{p}_{Si}^{(m)}(r) = \frac{B_i^{(m)}(r)}{N-m}, \;\; i = 1, \cdots, N-m, \;\; \text{for } SampEn,$$

Similar formulations are presented for m+1:

$$\hat{p}_{Ai}^{(m+1)}(r) = \frac{C_i^{(m+1)}(r)}{N-m}, \;\; i = 1, \cdots, N-m, \;\; \text{for } ApEn;$$

$$\hat{p}_{Si}^{(m+1)}(r) = \frac{C_i^{(m+1)}(r)-1}{N-m} = \frac{A_i^{(m)}(r)}{N-m}, \;\; i = 1, \cdots, N-m, \;\; \text{for } SampEn;$$

From these, **ApEn** is defines as:

$$\hat{A}pEn(m,r,N) = \hat{\Phi}^{(m)}(r,N) - \hat{\Phi}^{(m+1)}(r,N)$$

Where:

$$\hat{\Phi}^{(m)}(r,N) = \frac{1}{N-m+1} \cdot \sum_{i=1}^{N-m+1} \ln\left(\hat{p}_{Ai}^{(m)}(r)\right)$$

While **SampEn**:

$$\hat{S}ampEn(m,r,N) = \hat{\Psi}^{(m)}(r,N) - \hat{\Psi}^{(m+1)}(r,N)$$

Where:

$$\hat{\Psi}^{(m+1)}(r,N) = \ln\left(\frac{1}{N-m} \cdot \sum_{j=1,j\neq i}^{N-m} \hat{p}_{Si}^{(m+1)}(r)\right)$$

ApEn is given by the logarithmic sum, regarded as the informative content of the template.

SampEn, instead, is given by the matching probabilities average logarithm, regarded as the content of information of the average template of the series.

So, the first gives average information derived by matching probabilities, while the latter implements the average directly on the matching probabilities and then extrapolates information.

ApEn and SampEn are not immune to any estimation errors, as they involve the sum of many probabilities, which propagate their uncertainties.

Recordings' length affects both indexes: with short time series epochs, ApEn results underestimated, even enlarging the tolerance; SampEn, on the other hand, seems to be in accordance with its theoretical values, but when the results are averaged, the standard deviation happens to drastically increase affected by the duration of the time series.

The results might be made unstable also by zero-matches: they are equivalent to the self-match for ApEn, but, for a low threshold value and for a short time series, when no other pattern is found, SampEn might turn undefined because of the logarithm of zero.

Φ in ApEn adds the errors progressively with a logarithmic function, without error annulment. On the opposite, errors accumulate.

$$\hat{\Phi}^{(m)}(r,N) = \Phi^{(m)}(r,N) + \frac{1}{N-m+1} \cdot \sum_{i=1}^{N-m+1} \ln\left(1 - \frac{\varepsilon_{Ai}^{(m)}(r)}{p_i^{(m)}(r)}\right)$$

SampEn, instead, shifts the error distribution, making it symmetrical to the zero, and introducing error cancellation.

$$\hat{\Psi}^{(m)}(r,N) = \ln\left(\sum_{i=1}^{N-m} p_i^{(m)}(r) - \sum_{i=1}^{N-m} \varepsilon_{Ai}^{(m)}(r)\right)$$

In the end, through analysis both ApEn and SampEn showed to be affected from quantization errors and for both perks and disadvantages can be found. When a signal has a symmetrical error

distribution, SampEn is the most stable measure, is the time series is short. When the signal duration increases, though, ApEn seems to perform better, with the same results that would be reported by SampEn, but with lower standard deviation levels. On the other hand, starting from an asymmetrical error distribution, SampEn is the measure affected by the zero-matching, while ApEn performs regularly, as long as the time series is of sufficient duration [55].

Chapter 6

Phase-Amplitude Coupling

6.1 Introduction

Phase-Amplitude Coupling (PAC) is the proven phenomenon for which different neural cores across the whole brain communicate and influence each other because of the phase and the amplitude of distinct frequency oscillations. Several issues are found when tried to apply PAC to EEG, but there are different possible algorithms that can be utilized.

EEG signal can be divided into bands (delta, theta, alpha, beta and sometimes even gamma is considered) depending on the considered frequency range. Each of the bands is related to specific functions that can be related in a coupling pattern named "**Cross-Frequency Coupling**" (CFC). PAC represents one of the possible forms of CFC study where the phase of the lower frequency bands (Delta and Theta) influences the amplitude or the power of an oscillation in the higher (usually Beta or Gamma) ones. PAC studies can be applied to various brain regions, such as visual, auditory and prefrontal cortices, and hippocampus. The analysis can be performed with EEG, ECoG and MEG.

Alpha phase results strongly coupled to gamma amplitude in the visual system, as the latter gets segmented by the activity of the former. This coupling has been shown to depend on a brain coordination mechanism acting over various cognitive networks. Information might be tracked around different brain areas, then segmented or maintained.

PAC studies were also carried out on neurological patients like schizophrenic, Parkinsonian and autistic ones, showing interesting coupling patterns.

Correctly approaching to PAC studies is crucial to avoid false positives and artifacts effects, as there are different available algorithms capable to detect the coupling.

First of all, a proper filter and the Hilbert transform need to be applied to the time series to obtain the low frequency phase and the high frequency amplitude. The bandwidth needs to match at least the center frequency +- the modulating low frequency phase in order to for the PAC to be detected [57].

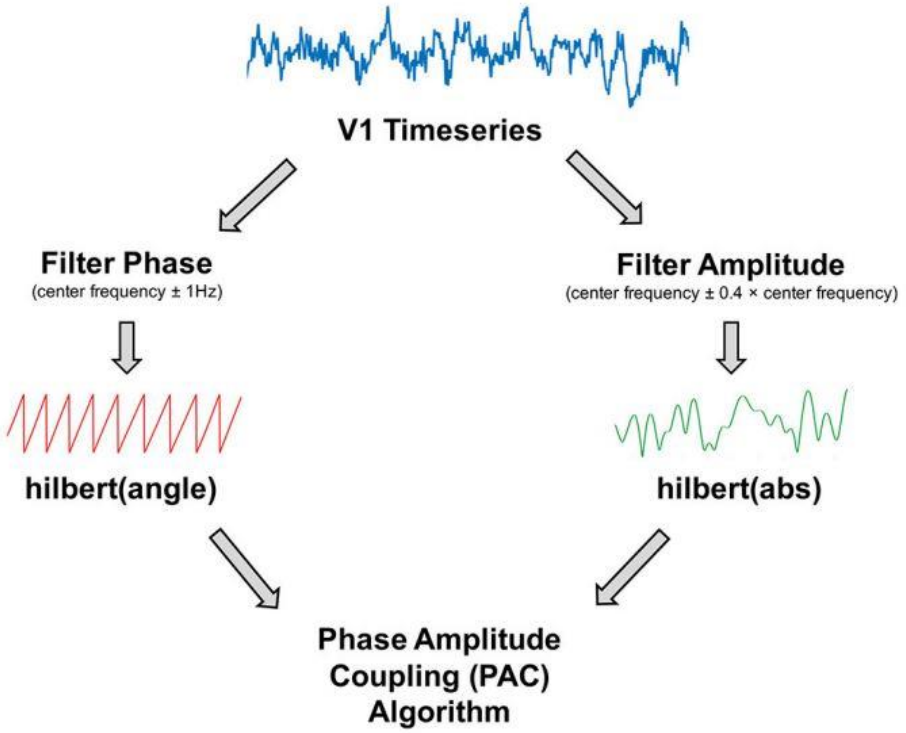


***Figure 20**. Phase-Amplitude Coupling (PAC) procedure: proper filters are applied to the time series and then Hilbert transform gives the Phase and Amplitude information necessary to apply the desired PAC algorithm [57].*

Some of the main PAC algorithms involve the following indexes:

- **Mean-Vector Length** modulation index (MVL)
- **Vector Length** modulation index
- **Kullback-Lieber** modulation index (KL)
- **Envelope-to-Signal** Correlation (ESC)
- **Phase-Locking Value** (PLV)
- **Generalized linear modelling** cross-frequency coupling (GLM-CFC) [57].

6.2 Mean-Vector Length modulation index

MVL combines a signal's amplitude and phase to create complex signal, $[\mathbf{f_a(n)\, e^{i(\Phi fp(n))}}]$, where each vector represents a point in time. The coupling between the low phase and the high amplitude can be computed taking in account the average vector length if the probability distribution function is non-uniform:

$$MI = \left| \frac{1}{N} \sum_{n=1}^{N} \mathrm{f}_a\,(n)\, e^{i(\phi \mathrm{f}_p(n))} \right|$$

This is not the only formulation of MVL, as there is a **normalized** version which involves the power of the high amplitude. In fact, MVL was proven to represent the high amplitude oscillation's power more than the coupling, so the power value was chosen as the normalization factor.

$$MI = \frac{1}{\sqrt{N}} \frac{\left| \frac{1}{N} \sum_{n=1}^{N} \mathrm{f}_a(n)\, e^{i(\phi \mathrm{f}_p(n))} \right|}{\sqrt{\frac{1}{N} \sum_{n=1}^{N} \mathrm{f}_a(n)^2}}$$

6.3 Phase-Locking Value

PLV starts from the premise that PAC leads to the high amplitude envelope to oscillate at the lower phase frequency. The high amplitude envelope frequency is given by the Hilbert transform and then the PLV is computed as the phase synchronization between the signals:

$$MI = \left| \frac{1}{N} \sum_{n=1}^{N} e^{i(\phi \mathrm{f}_p(n) - \phi \mathrm{f}_a(n)} \right|$$

6.4 Kullback-lieber modulation index

KL quantifies PAC as the deviation in the distributions of phase and amplitude. A certain number of bins separate the low phase frequencies and then the amplitude is averaged for each phase bin. The bin number is not fixed and can be chosen as it does not affect the results. A null hypothesis is made of a uniformly phase-amplitude distribution, and the modulation index is obtained comparing the real distribution to the hypothetic one.

$$MI = \frac{D(P, Q)}{\log(N_{bins})}$$

The distribution is calculated with the Kullback-Lieber distance, based on Shannon's entropy:

$$D(P, Q) = \sum_{i_{bin}=1}^{N} P(i_{bin}) \cdot \log\left(\frac{P(i_{bin})}{Q(i_{bin})} \right)$$

6.5 Generalized linear modelling cross-frequency coupling

GLM coupling tries to find a mathematical model capable to relate observed variables. The low frequency analytic signal gives the phase and from the high frequency one the amplitude is extracted. In addition, the latter is real and positive and in a non-linear relationship to the phase. Using a scatter plot for phase and amplitude, PAC can be visualized if the amplitude values result higher in specific phase values. PAC won't be present, instead, if the amplitude values appear similar through all the phase possible values. The data can be modelled with a horizontal line called null model when there is no phase-amplitude coupling, and with a curve known as spline whenever PAC is present. In general, the model can be regarded as a spline similar to the null model in the case of no-PAC, and then it gradually curves as the coupling increases in the data. The maximum absolute difference between the two models is used to calculate the percentage change. The curve can be considered as the union of multiple 3rd order polynomial splines scattered upon a certain number of control points. The user can freely set the number of control points, as a parameter which can affect the final results [58].

6.6 Envelope-To-Signal Correlation

Envelope-to-signal (ESC) correlation is a strongly complex PAC measure based on demodulation of signals. Thus, it acts on the analytical signal's amplitude and phase, and it is heavily influenced by the bandpass filter used in the demodulation. Centers of frequency are in fact chosen, and the same filter width is set for every frequency center. The centers are selected at fixed steps in between of frequency range that must be analyzed, while the filter's width is decided at reasonable values compared to the whole frequency range.

ESC correlation is calculated as a cross-correlation between the envelope of a signal at high-frequency and the signal at a low frequency. This might be done at the same recording location or at others. It must be noted that ESC correlation is different from Envelope Correlation, which instead computes the cross-correlation between envelops. ESC correlation is quite suggested for its Cross-Frequency-Coupling applications, for its consistency in measuring cortical interactions with non-linear couplings [60].

6.7 Comparisons

Cross-frequency coupling is strongly affected by sawtooth non-sinusoidal oscillations which enormously alter PAC results. The ratio between rise and decay of the oscillations allows to determine if the PAC results are due to the non-sinusoidal properties or not.

There is evidence of alpha-gamma coupling in the occipital cortex resulting from stimulation. Alpha relative power seemed to decrease, while the gamma one increased. When stimulation is involved, the detected coupling pattern change depending on the chosen algorithm.

Another problem could be due the trial length, as using trials shorter than 10 seconds could interfere with theta-gamma coupling detection. This is not the case of alpha-gamma coupling, because even 1-second-long epochs are sufficient to gain stable results.

A common mistake in PAC analysis occurs when considering the extreme points of a time series which has been bandpass filtered. These points could lead to edge artifacts, so zero-padding might be necessary. The same happens when different epochs are concatenated to create longer segments.
In addition, the filter bandwidth needs to contain the side band of the phase frequency, or the PAC can not be detected. This is typically overcome by using a variable moving band-pass filter.

Non-stationary sections of the signal should be avoided, such as for sensory evoked potentials because they induce a frequency correlation misinterpreted as PAC.

Pac values range from 0 to 1, so the resulting data are usually not normally distributed and need to be analyzed through non-parametric statistics.

The main limitation of PAC studies relies in the many non-sinusoidal oscillation which occur in the physiology and pathology of the brain. For this reason, spurious PAC could lead to interesting results, but the main PAC analysis methods are not best suited for this kind of applications, even though there are many other available PAC algorithm that can be employed.

MVL results to be the most stable index when the signal trials have at least a certain duration, high sampling frequency and suffering from little noise. Whenever the signal should be affected by too much noise, recorded at low sampling frequencies and arranged in short epochs, other indexes such as KL's are recommended. Furthermore, the cross-frequency coupling can be monophasic and biphasic, and KL's modulation index is suggested if this information is not known. KL's algorithm is based on Shannon's Entropy which depends on the bins number. The choice of this parameter could lead to different results. This is not the case for MLV, though,

Both MLV and KL modulation indexes are usually computed to make up for each other's flaws, even though the computational time might result extended. This is not to be overlooked as performing PAC requires heavy computations which could last many hours even on the best devices.

PLV, on the other hand, does not show great results speaking of PAC. This is due to the phase information, which is extracted from the signal's amplitude envelope, while it should correctly be obtained from real oscillating signals. Using the right filters might overcome PLV's weakness. Moreover, PLV shows its best results when applied to graph theory.

General comparisons between all the CFC methods are not easily made especially if the analysis is made on different studies, in different conditions. Permutation testing can be carried out to work in standardized conditions, permitting better interpretation and comparisons [57] [58] [60].

Chapter 7
Functional Connectivity

7.1 Introduction

Brain's connectivity and its network patterns and organization can be studied applying a mathematical analysis approach to EEG named **Graph theory**. Linear analysis is not able to fully characterize EEG signals, while graph theory is able to map the functional connectivity of the Brain. The main goal is to understand the dependency of a brain region on the others, especially through statistical means. Graph theory can be applied to EEG signals in different manners, on different circumstances, ranging from cognitive tasks to sensorimotor ones. Many indexes can be employed to assess the functional correlation, each with their own advantages. These measures allow to recreate the brain network, which can be analyzed with specific metrics that differentiate global integration and local segregation.

Graph theory's main fields of study regard the brain's topological properties in particular in patients suffering from neurological disorders and dysfunctions, people at rest or subjects undergoing high cognitive tasks such as perception, memory or attention.

This theory was born in 1736 thank to Leonard Euler who hypothesized a "graph" as a mathematical structure made of "nodes" and "edges" just to solve a logic problem. This allowed him to exclude external features, focusing only on the useful information and their connections.

Even if in the last century graph theory has been utilized in electrical and chemical fields, it is only in the late 1990s that brain connectivity patterns quantification has begun to be studied through it. This was enabled by the discoveries made for modelling the network, which lead to pursuing brain's topological characterization trough segregation and integration [61].

7.2 Connectome

Different brain region's connectivity and the way the diverse regions communicate is called connectome. There are three separated yet also related types of connectivity:

- **Structural connectivity**, based on the neurons' physical connections. It is referred to the brain's white matter connectivity;

- **Functional connectivity**, regarding the dependencies between time series deriving from diverse regions of the brain, from a statistical point of view;

- **Effective connectivity**, which analyzes one neural element's causal effect and influence on other neurons.

While the first one has a more neuroanatomical meaning, the last two are based on the time series recorded at different time points, from different brain regions.

The networks models are built starting from the data deriving from two main neuroimaging tools: EEG and fMRI. EEG has been employed for its non-invasiveness, portability and temporal resolution, whereas fMRI had better spatial resolution, lacking in time resolution.

High time resolution appears to be preferably when cognitive processes and information flows are studied. EEG was, in fact, preferred and employed for the first graph theory applications on patients with Alzheimer's disease. EEG indeed has the highest time resolutions, reaching values inferior to 1 millisecond, perfectly capable to identify rapid neuronal activity.

Functional connectivity relies on estimators with specific characteristics. There are many and they are categorized according to different characteristics such as:

- Number of time series involved (**univariate** or **multivariate**)
- Direction of the calculation (**directed** or **undirected causality**)
- Domain (**time** or **frequency**)
- Presence of phase coupling
- Statistical dependency (**linear**, **non-linear** or **information-based**)
- Volume conduction sensitivity

When using a specific neurological analysis technique on a single signal, univariate analysis is suggested, while the multivariate one should be adopted when different techniques are combined.

Linear, non-linear and information-based techniques are used according to the linearity level of the phenomena in question, and to assess causality between time series and then brain regions.

Functional connectivity measures are composed of combinations of the previous listed characteristics, and it is difficult to choose the proper one, or ones, for the analysis [61].

Some of the most used correlation measures are the following:

- **Magnitude squared coherency**
- **Cross-correlation**
- **Phase locking value**
- **Phase lag index**
- **Mutual information**
- **Transfer entropy**
- **Generalized synchronization**
- **Granger Causality**

7.3 Graph theory

Graph theory is based on a topological representation of a certain number of **vertices** (V), also known as **nodes**, connected through **edges** (E). This representation is called **Graph** (G):

$$G = (V , E)$$

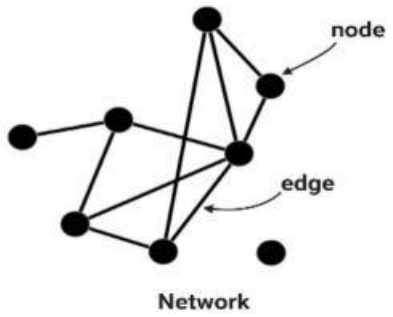


***Figure 21**. Example of a Graph Network, showing its Nodes and Vertices [61].*

From a practical point of view, when the subject of interest is the brain, its regions are represented by the nodes. As brain region, a proper anatomical portion is meant, or just the EEG electrode zone. The edges, instead, depend on the connectivity study, as they can represent anatomical connections or statistical measures. Time series correlation measures between nodes are considered in functional connectivity. A graph's edge can be directed or undirected depending on whether the correlation direction is relevant or not. In particular, direct edges imply causal influence for which the information flowing from a node to the other goes in only one direction. This way, every node's activity is dependent from the activity of the one which sent the information in the first place. Indirect edges, instead, connect nodes without a specific preferred direction, allowing information to flow in either way. Graphs can also be weighted or unweighted, according to strength of the edges. Weighted graphs differentiate the connections into strong and weak ones, associating a value to each. Setting a threshold allows to eliminate the weakest connections. On the other hand, unweighted graphs do not associate any kind of strength to the connections, as they have all the same value, if present [61] [64].

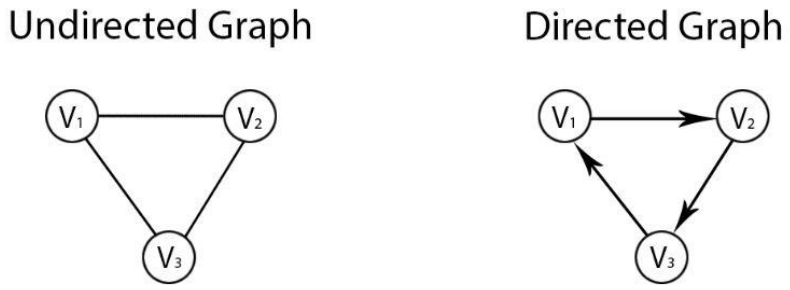


***Figure 22.** Undirected vs. Directed Graph representation [61].*

7.4 Pipeline for a functional connectivity brain network construction based on EEG

Once the main components of a graph have been discussed and since EEG has been selected as the best candidate for functional connectivity applications, the pipeline steps necessary to create the brain network are presented [61]:

1. **Brain network nodes definition**
2. **EEG data pre-processing**
3. **Edges definition**
4. **Connectivity matrix computation**
5. **Connectivity matrix binarization**
6. **Thresholding**
7. **Functional connectivity measurement estimation**
8. **Network construction**
9. **Graph theory metrics computation and application**
10. **Statistics application**
11. **Classification**

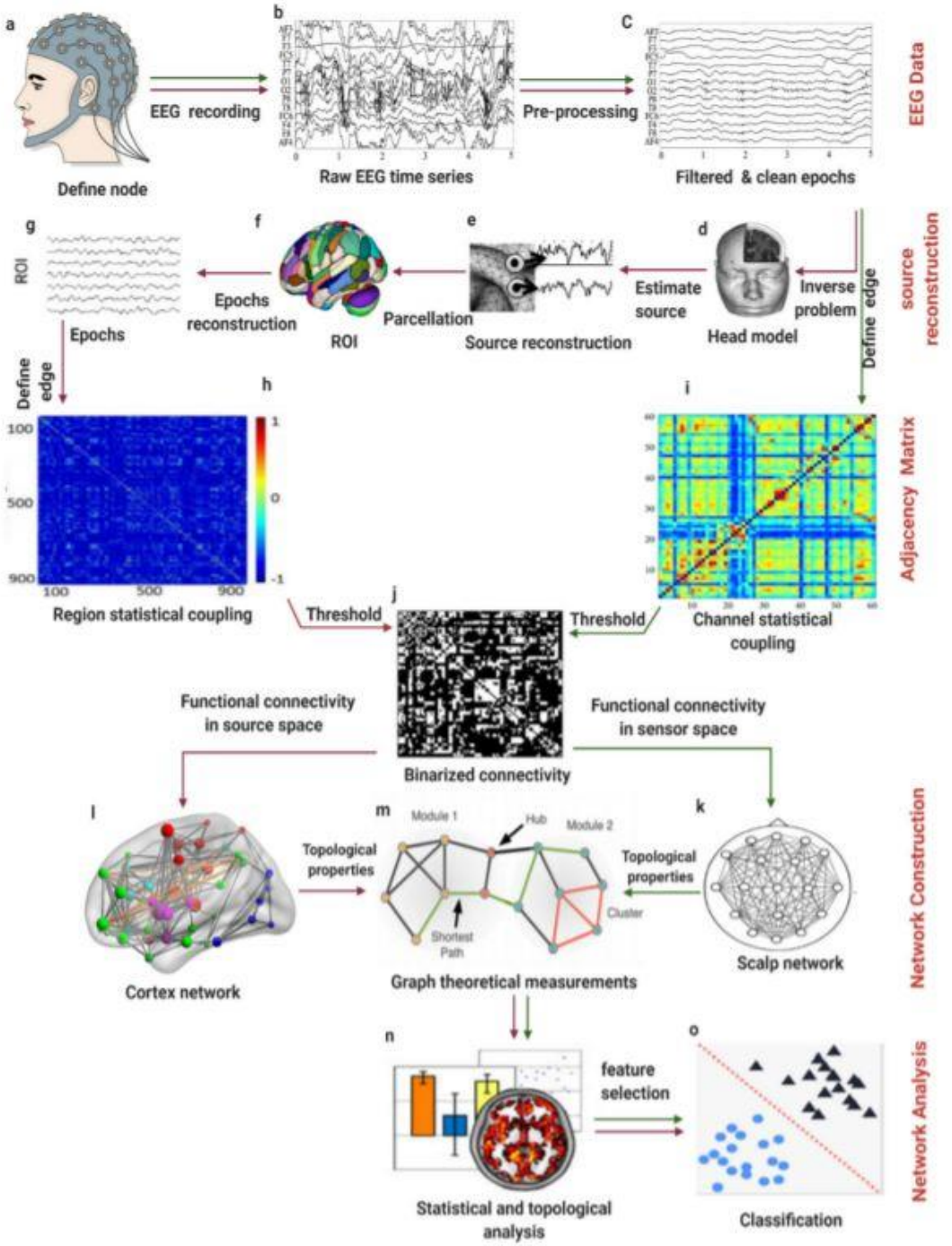


Figure 23. *Pipeline for a functional connectivity brain network construction based on EEG: a) Brain network nodes definition; b) EEG data acquisition; c) Data filtering and pre-processing; d) Head modelling in inverse process; e) Reconstruction of the source; f) Parcellation to Regions Of Interest (ROI); g) Epochs reconstruction for each ROI; h) Correlation across ROIs; i) Correlation across channels; j) Binarization of the correlation; l), m), k) Network reconstruction; n) Statistical analysis of functional measures; o) Classification [61].*

7.4.1 Brain network nodes definition

There are two approaches when nodes are defined in EEG studies: the first one consists in the association of each node to a single EEG electrode; the second one involves larger areas but is also more complex to set. In this case, the brain is subdivided into regions of interest according

to its anatomical and functional portions. This is implemented with parcellation schemes deriving from brain atlases.

In both cases, EEG is acquired, but in the first one each channel is simply associated to a node, while in the second the tridimensional source of the signal is reconstructed through the solution of an inverse problem based on dipole theory.

The inverse problem starts by modelling the head, with simple spherical models of with a more realistic model obtained through imaging, like MRI. The source localization in the head model is then implemented by determining the dipole source that reconstructs the time series with an algorithm. The reconstructed signal is then divided and parcellated into the regions of interest of the brain, obtaining a regional time series [61] [64].

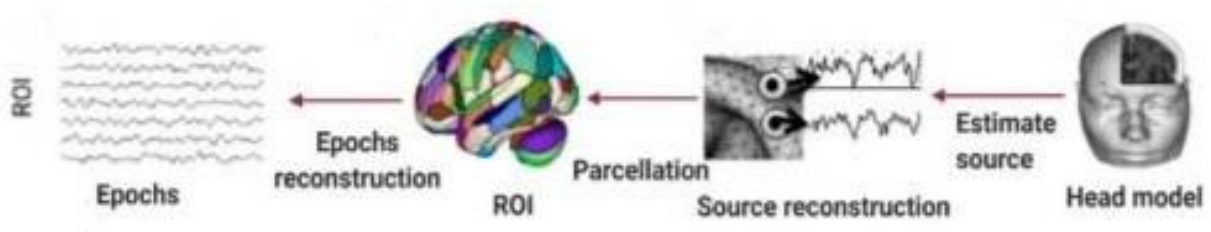


***Figure 24**. Inverse problem of reconstructing regional time series for specific regions of interest starting from acquired EEG and a head model [61].*

7.4.2 EEG data pre-processing

The acquired raw EEG signals can't be used as such because pre-processing is needed. First, it must be segmented and filtered. EEG is affected a lot by noise and artifacts which must be removed with the use of the right filters and techniques. In addition, working with signals comparable to the quasi-stationary state is preferably, so the time series are divided into clean and stationary epochs. At this point, in addition, different frequency ranges can be extracted from the signals because they might be more informative if analyzed a band at a time, rather than together [61] [63].

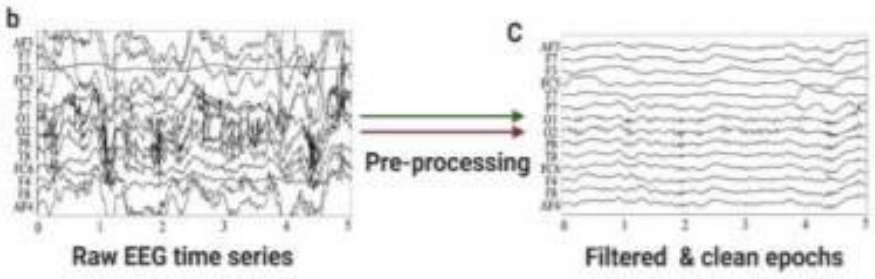


***Figure 25**. Portion of the Pipeline highlighting the Pre-processing needed for Raw EEG time series [61].*

7.4.3 Edges definition

The nodes can be connected through different connectivity patterns, but the connection's characteristics (direct/indirect, weighted/unweighted, time/frequency domain) are determined when the correlation coefficient used to represent the edge is chosen.

7.4.4 Connectivity matrix computation

The connectivity matrix is also known as adjacency matrix (A). It is an **N x N matrix** for which the **i** and **j** elements represent the nodes, and the **aij** entries the edges. In substance, element aij represents the correlation given by a correlation coefficient between node i and node j. It is a weighted matrix, and it is symmetrical for undirected networks [61] [64] [66].

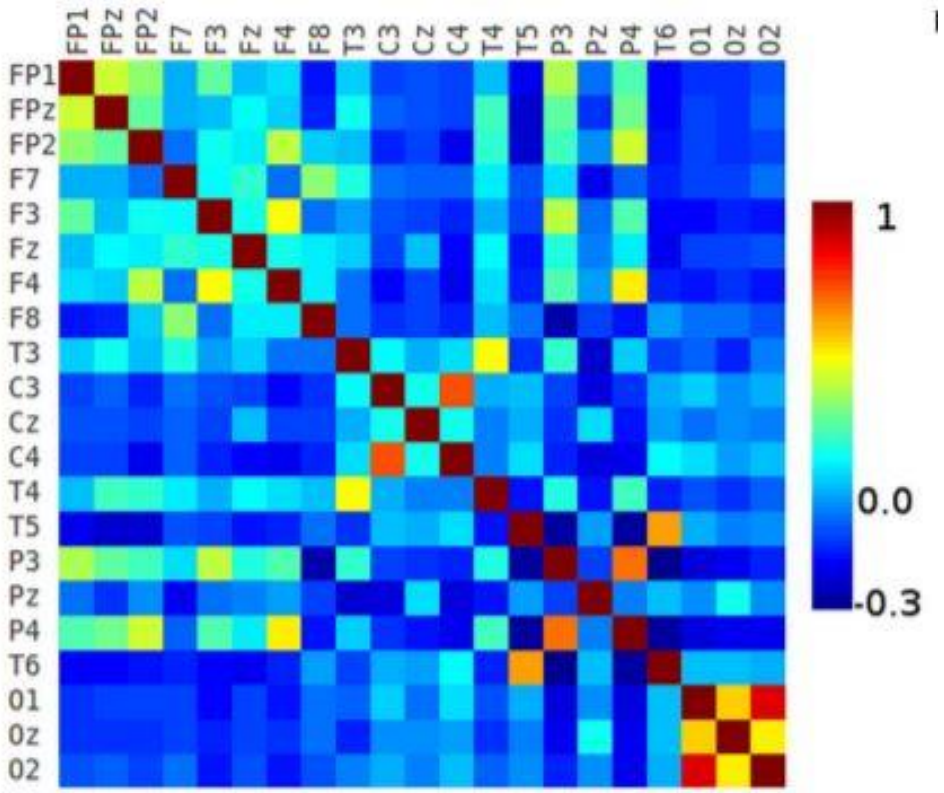


Figure 26. *NxN Connectivity (or Adjacency) Matrix: N is the number of nodes, in this case represented by the electrodes [61].*

7.4.5 Connectivity matrix binarization

Correlation coefficients usually report a certain numeric value to the correlation between node pairs. This value might be higher or lower depending on the strength of the correlation. When all the correlation coefficients are calculated the adjacency matrix can be completed. Sometimes, though, it is not necessary to quantify the correlation, but instead all it is need is to be able to say if the correlation is present or not. So, a certain **threshold** can be applied to the adjacency matrix which puts to 0 all the elements below and sets to 1 all the ones above, allowing to discriminate

if the correlation for which node pairs the correlation is present. An unweighted adjacency matrix is this way obtained [61] [64] [66].

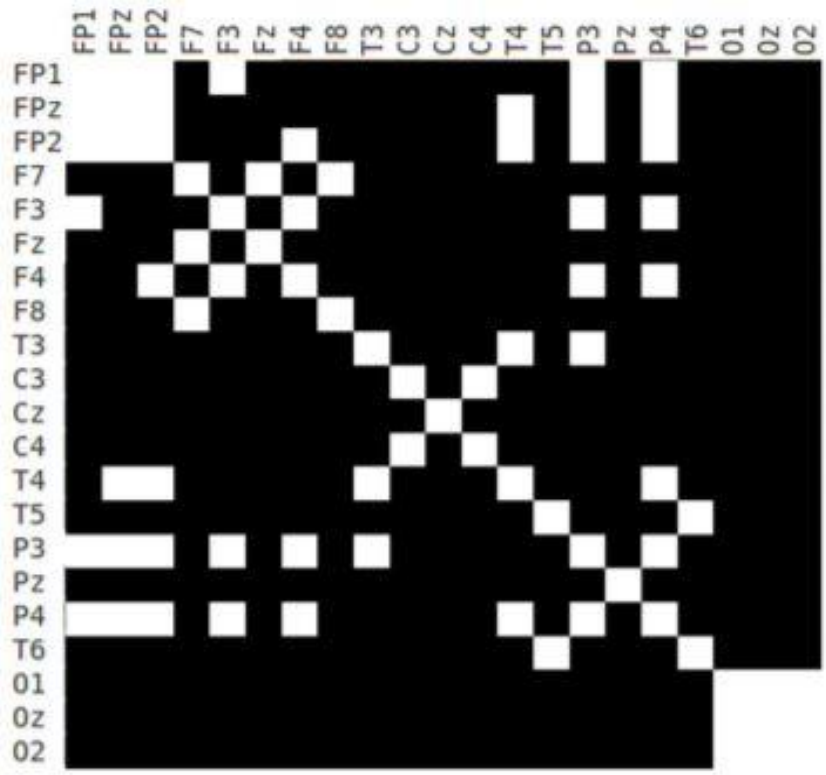


Figure 27*. Binarized Adjacency Matrix [61].*

7.4.6 Connectivity matrix thresholding

The starting weighted adjacency matrix can be submitted to another kind of threshold: this threshold leaves unaltered the correlations above, while still putting to zero the values below. It is useful to simplify the complexity of the network because weak and noisy edges are removed. The null model can then be easily defined for statistical analysis. The main issue is to establish the right threshold value because even small differences can enormously affect the network's properties and results. Many thresholding criteria and algorithms are available, but the preferred ones are those which control and minimize type-1 errors occurrences [61] [64] [66].

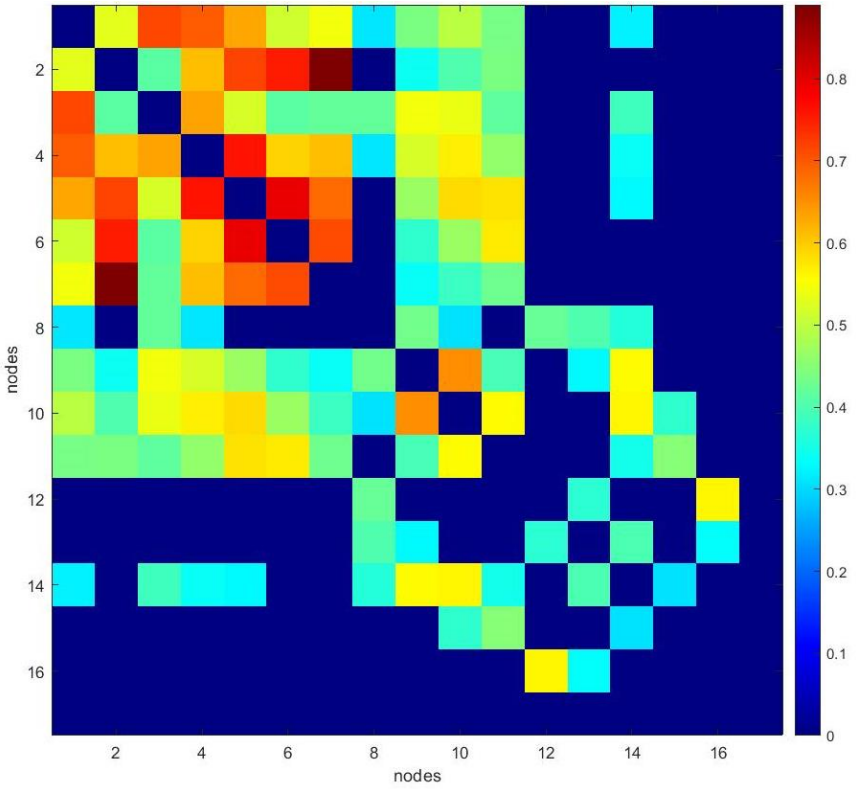


Figure 28. *Thresholded weighted Adjacency Matrix [61].*

7.4.7 Functional Connectivity measurements estimation

Many functional connectivity measures are available to be compared, but there is not a precise technique that can be defined as optimal. The connectivity estimator choice relies on many factors. First, the studied hypothesis must be defined. The choice will then depend on the linear, non-linear or information-based interdependencies between nodes, the time or frequency domain

In general, indexes that measure in the frequency domain result to be more suited to EEG signal because of their consistency in separating actual signal from artifacts and because of frequency bands analysis. Multivariate analysis has been supported for its results, and lastly, non-linear analysis has been suggested thanks to its sensitiveness towards non-linear couplings in EEG time series [61] [62] [65] [66].

Functional estimator	Univariate	Multivariate	Direct Causality based	Indirect	Time-domain	Frequency domain	Phase coupling	Linear	Non-linear	Info-based	Volume conduction sensitivity
Correlation	✔			✔	✔			✔			Highly sensitive
Cross correlation		✔	✔		✔			✔			Less sensitive
Magnitude squared coherence [70]	✔			✔		✔		✔			Highly sensitive
Phase locking value (PLV) [71]	✔			✔		✔	✔		✔		Highly sensitive
Phase lag index (PLI)	✔			✔		✔			✔		Less sensitive
Weighted phase lag index (wPLI) [72]	✔			✔		✔			✔		Less sensitive
Partial coherence		✔		✔		✔			✔		Robust
Mutual information [73]	✔			✔	✔					✔	Robust
Transfer entropy	✔		✔		✔				✔		Less sensitive
Generalized synchronization		✔		✔						✔	
Synchronization likelihood [74]	✔			✔					✔		Sensitive
Phase synchronization		✔		✔			✔		✔		Sensitive
Granger causality [75]		✔	✔		✔	✔		✔			Less sensitive
Directed transfer function (DTF) [67]		✔	✔			✔		✔			Sensitive
Imaginary part of the coherence [76]	✔			✔		✔		✔			Less sensitive
Partial directed coherence (PDC) [77]		✔	✔			✔		✔			Less sensitive

***Table 2**. Functional Estimators listed and categorized according to the characteristics they might or might not satisfy, simplifying the choice of the most suited to different kinds of analysis [61].*

7.4.8 Network construction

The connectivity matrix allows to reconstruct the brain network. This can be visualized on a scalp model or on a tridimensional head model. The network is given by the transposition of the connectivity matrix's values, binarized or not, to the nodes which correspond either to brain regions, or to EEG electrodes.

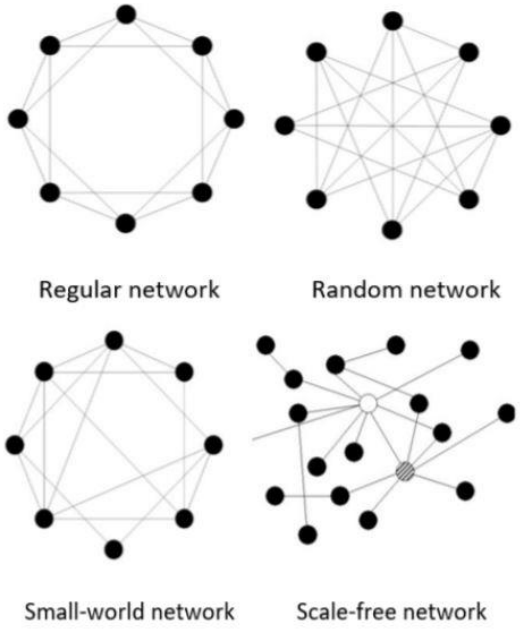


***Figure 29**. Types of Graph Networks, resulting from the number of local segregations and the global integration between nodes, both given by FC metrics [61] [63].*

7.4.9 Graph theory metrics computation and application

Once the connectivity matrix has been created and the network has been constructed, different metrics can be used to quantify the topological properties of the nodes and the network itself. These features measure local segregations and the network's integration, allowing to establish the quality of the network in terms of robustness and efficiency to transfer information: **Regular Networ**ks, for example, are robust but not efficient in information transmission; **Random Networks**, on the opposite, are efficient in transferring information but lack in robustness; **Small-world Networks**, instead, represent intermediate cases, optimal in terms of cost, integration and segregation, while **Scale-Free Networks** are quite unique because of their short paths. In detail, robustness is given by the Clustering Coefficient metric, while the efficiency in information transferring is given by the Path Length metric. Nevertheless, there are many other FC metrics that, once integrated, describe the network at their best [61] [63].

7.4.10 Statistics application

The calculated FC metrics are used to the describe the network itself, but also for comparisons across populations, states or conditions, or between results and theoretical references. The topological properties are assessed through significance statistical means. Confidence intervals are applied to measure the results' significance and the graphs' reliability. Non-parametric and permutational statistics are also employed, especially for their adequacy for EEG data. Since the resulting features deriving from graph theory are countless, usual statistics has failed to investigate the characteristics of connectomes. Automatic algorithms of graph detection have been developed to overcome this obstacle, using hierarchical Bayesian and Gaussian graphical models [61].

7.4.11 Classification

Graph theory allows to go beyond comparing conditions, pathologies or populations. FC measures can be used to classify different conditions according to several methods. Classification can be manually achieved by comparing the results with prototypes, or either with automatic algorithms that take advantage of artificial intelligence (A.I.), such as Machine Learning and Deep Learning [61].

7.5 Functional Connectivity Correlation Measures

There are several correlation measures that can be calculated between the nodes of the graph, even though they present different perks and limits from each other. The possible characteristics of said measures have been listed in the pipeline for functional connectivity brain networks construction, but the main ones are discussed in detail as follows.

7.5.1 Cross-Correlation

Cross-Correlation is a correlation index usually used to assess the delay in time between two time series. It represents the best correlation tool when analyzing signals in time domain because, independently from eventual delays, it is capable of finding signal similarities. In this way, it can be employed to investigate signals deriving from different EEG electrodes giving sensitive and accurate results, without being affected by the signals' amplitude. In this case, cross-correlation represents a measure of how much two signals are close and related, both if they are measured at the same time than considering time delays. **Cross-correlation** for two time series, in its normalized form, is defines as:

$$R_{xy}(\mathcal{T}) = \frac{\frac{1}{N}\sum_{t=1}^{N-\mathcal{T}}[(x_t - \mu_x)(y_{(t+\mathcal{T})} - \mu_y)]}{\sigma_x \sigma_y}$$

Where $\boldsymbol{\mathcal{T}}$ represents the time delay, **N** the signals' length, **μ** the mean of a signal and **σ** the standard deviation of a signal. The lengths of the signals can be different, and the values of R range between -1 and 1, respectively for inverse and exact matchings [70] [71].

7.5.2 Mutual Information

Mutual information (MI) is a correlation index deriving from information theory. It is used to evaluate the amount of information of a signal contained in another time series. It is a statistical measure which does not take in account any characteristics of the signal's nature, such as linearity, and can be used even with long signals. It is widely used to assess measures between signals in narrow frequency bands, making it an adequate option for EEG signals. In this case, it can be used as a tool to quantify correlations between EEG's bands, distinguishing different states and conditions across brain regions. Mathematically, it measures dependencies, both linear and non-linear, between two signals. It

performs like the counterpart of the correlation between time series, which indeed identifies their linear dependency. MI is also known as **Auto Mutual Information (**AMI) when referred to a single signal, and **Cross Mutual Information** (CMI) when applied to a pair of data series.
MI's formulation is the following:

$$MI(X, Y) = H(X) - H(X|Y) = H(X) + H(Y) - H(X, Y)$$

Where:

$$H(X) = -\sum_{x_i} P_X(x_i) \log P_X(x_i)$$

$$H(X \mid Y) = \sum_{y_j} P_Y(y_j) H(X \mid Y = y_j) = -$$

$$\sum_{x_i, y_j} P_{XY}(x_i, y_j) \log \left(\frac{P_{XY}(x_i, y_j)}{P_Y(y_j)} \right) = H(X, Y) - H(Y)$$

Here, **X** and **Y** represent the data sets, **Px** and **Py** the probability distribution of amplitudes, **H(X)** and **H(Y)** the average information given by X and Y from Shannon's theory of information, and **H(X|Y)** is the average conditional uncertainty given by the measure of X, knowing Y.
So, MI measures the information of a signal measuring the other, quantifying the uncertainty reduction on a data set provided by measuring the other [72].

7.5.3 Covariance

Covariance measures the simultaneous changes of two signals. Mathematically, it gives information about the signals' changes in respect to their mean values. It does not represent dependencies, but it tells whether the two are changing at the same time or in the same manner. Positive values indicate changes in the same direction. When calculated on a single signal, covariance coincides with variance. Covariance between two signals is defined as follows:

$$\mathrm{Cov}(X, Y) = \frac{\sum (X_i - \bar{X})(Y_j - \bar{Y})}{n}$$

It is usually computed as a matrix. Given a couple of signals, the matrix's dimensions are 2x2, where the variance of the single signals is found on the main diagonal, expressing how the information related to a single channel is varying over time, while the relation between the two signals, affecting each other over time, in the form of covariance, is in positions (1,2) and (2,1) [73].

7.5.4 Pearson's Correlation Coefficient

Pearson's correlation is a tool that starts from covariance but is able to determine the strength of the connection between two variables, in addition to its direction. In fact, it measures the linearity of a correlation [74]. The coefficient is defined as follows:

$$r_{XY} = \frac{E\left[\left(X - E(X)\right)\left(Y - E(Y)\right)\right]}{\sigma_X \sigma_Y} = \frac{E(XY) - E(X)E(Y)}{\sigma_X \sigma_Y}$$

With **E** representing the expected value and **σ** the standard deviation. Its values range in the [-1,1] interval. Since a signal is a time series composed of n samples, Pearson's correlation coefficient can be indicated as:

$$r_{XY} = \frac{1}{n-1}\sum_{i=1}^{n}\left(\frac{x_i - \bar{x}}{\sigma_X}\right)\left(\frac{y_i - \bar{y}}{\sigma_Y}\right) = \frac{\sum_{i=1}^{n} x_i y_i - n\bar{x}\bar{y}}{(n-1)\sigma_X \sigma_Y}$$

7.5.5 Phase Locking Value

The Phase Locking Value (PLV) is a correlation measure that reflects the phase synchronization between signals. Generally, it is associated with coherence, even though PLV's performances have been proved to be more consistent. In any case, PLV is calculated starting right from coherence, but applied to Fourier transformed signals with normalized amplitude:

$$plv_{xy}(\omega) = \frac{\left|\frac{1}{n}\sum_{k=1}^{n} 1_x(\omega,k)1_y(\omega,k)e^{i\left(\varphi_x(\omega,k)-\varphi_y(\omega,k)\right)}\right|}{\sqrt{\left(\frac{1}{n}\sum_{k=1}^{n} 1_x^2(\omega,k)\right)\left(\frac{1}{n}\sum_{k=1}^{n} 1_y^2(\omega,k)\right)}} = \left|\frac{1}{n}\sum_{k=1}^{n} e^{i\left(\varphi_x(\omega,k)-\varphi_y(\omega,k)\right)}\right|$$

Coherence alone results affected by amplitude correlations, possibly misleading the interpretation, while PLV gives appreciable values separating phase synchronization and amplitude correlation [75].

7.5.6 Phase Lag Index

Phase Lag Index (PLI) is another correlation measure based on the signals' phase. Its main target is to overcome the effects of volume conduction and active reference electrodes given by common sources. This is achieved by discarding the phase differences that are centered around 0 at every π. Whether phase synchronization is present or not is determined by the distribution of phase differences between the signals around zero. The asymmetry of the phase lag distribution can't be explained by volume conduction and active electrodes because their effect would be instantaneous.
PLI is given by the following formulation:

$$PLI = |\langle \mathrm{sign}[\Delta\phi(t_k)] \rangle|$$

PLI's values range from 0 to 1, where 0 stands for absence of coupling or coupling precisely at 0 mod π (volume conduction), while 1 is for perfect phase synchronization at a Δφ value different from 0. Whether one or the other signal is leading in phase is not indicated by this formulation of PLI, but this information can be obtained by removing the absolute value.
PLI reduces Type I errors by being insensitive to volume conduction identifying it not as a connection. On the other hand, Type II errors might occur because of missed connections due to too small lags [75] [76].

7.6 Correlation Measures interpretation problems

There are several problems that might affect connectivity measures and lead to issues for their interpretation. The main ones are listed and discussed as follows:

1. **Common reference problem**:
   This problem appears whenever a common reference channel is used for EEG recordings. The electric potential difference between the electrode and the reference is depicted by each signal, with all its fluctuations. These ones are consequently reflected to the correlation measure if the reference electrode is the same for all the electrodes. This leads to spurious correlation values at zero-time lag, depending on the dimension of the fluctuations of the potentials. The common reference problem can be avoided by adding channels recordings and performing bipolar derivations and computing the correlations between them locally referenced. Another solution is presented by referencing every channel in a separated way, eliminating coherence components.

2. **Volume conduction / Field spread**:
   This problem is mainly encountered with non-invasive EEG, because of the acquisition coming from the scalp. In fact, a single channel measures a current that has passed through different tissues before arriving to the scalp, with all the artifacts resulting from that. Field spread is so defined as the diffusion of electromagnetic fields due to neuronal sources that are recorded by multiple channels at once. This can lead to artifacts involving coherence and

phase locking, compromising the connectivity study. Volume conduction can be avoided with source reconstruction algorithms, using subtracting contrast experiments and choosing correlation metrics unaffected by interactions at 0 mod π, like PLI.

3. **Signal-to-noise ratio problem**:
   This problem starts from pointing out that separated signals might result into different correlation measures according to their amount of signal and noise. It is a condition strictly correlated to volume condition because of the common cause, but it must be noted that the signal-to-noise (SNR) problem might show up also in other circumstances.
   The problem could be mitigated by equalizing the impedance across all channels before even recording the EEG data, or by trying to maximize SNR through algorithms that separate the signal from the noise.

4. **Unobserved common input**:
   This problem assumes that interactions between channels might be detected despite the fact that it might come from another input. This happens because two signals are compared at a time, analyzing whether one might be cause of interactions over the other. The results change when the analysis is performed across a larger number of signals, because of the deriving combinatorial and statistical elements. In the FC study for EEG, the interaction causes are several, and several are the channels that must be compared. Sub-networking might help reducing the common input problem, permitting better results interpretation.

5. **Sample size bias**:
   Correlation measures result affected by the sample size of the observations or the epochs. Smaller sample sizes bring larger biases, which result in an overestimation of correlations when the sample size is reduced. Since different sample sizes across channels or epochs might thus give biased results, the problem might be reduced or even eliminated by comparing the same number of trials across channels. This can be achieved by randomly removing trials from the condition with the highest number, until the trial number coincides. This should be done multiple times in order to obtain more reliable results. Another approach consists in directly calculating the bias of a single FC measure, and then subtracting it from the correlation measure [62].

7.7 Functional Connectivity Metrics

Once the correlation measures have been computed and the connectivity matrices constructed, several metrics can be applied and calculated to the matrices to characterize the brain network. These metrics are subdivided into Global measures and Nodal measures: they respectively describe statistical characteristics and information of the whole network or of single nodes composing it. The main Global and Nodal measures are listed in *Table 3*, and some of them are discussed as follows:

Global measurement	Nodal (local) measurement
Characteristic path length (PL)	Local efficiency (Elocal)
	Nodal centrality
Global efficiency (Eglobal)	Degree centrality (K)
Clustering coefficient (CC)	Hub
Small-worldness (σ)	Degree distribution P(k)
	Degree correlation
Transitivity (T)	Betweenness
	Eigenvalue centrality
Network density (D)	Closeness centrality
Assortativity	Nodal efficiency (Enodal)
Modularity (Q)	Motif (M)
	Network cost

Table 3. *Global and Nodal Functional Connectivity measures [61].*

- **Characteristic Path Length** (PL): it is the main measure regarding global communication and functional integration of brain regions. It is given by the shortest averaged length of paths across all the network's nodes. Low PL values represent connection between brain regions that allow easier information flow.

- **Global Efficiency** (Eglobal): it measures the efficiency of the information flow through the brain. It is given by the inverse of the averaged path length. As an inverse of PL, higher Eglobal values represent higher integration and efficient multidirectional information transfer.

- **Transitivity**: it is given by the number of triangles found in the correlation matrix. It measures how triplets are embedded in the network.

- **Clustering Coefficient** (CC): it is the main measure regarding functional segregation, and it is given by the proportion of edges related to adjacent nodes, for every possible edge. With high CC values the network results more efficient and robust in its local interactions.

- **Local Efficiency** (Elocal): it represents the efficiency of a single node to transfer information to the closest ones. It is given by the average efficiency of all node pairs, for every single node.

- **Small-Worldness**: it quantifies the closeness of the network to a small-world kind of network. It is given by the ration of CC and PL, both normalized, and it tells the likelihood of nodes to be reached by every other one with a low number of steps.

- **Degree**: the network's degree is given by the ratio of the connections number in the network and its maximal number. Its values range between 0 and 1. Lower values indicate less connected networks, while higher values stand for steady networks. The Degree can also be calculated as a distribution across nodes, and it can be analyzed whether the degree of a single node is influenced by other nodes. Nodes connected to many others result to be more important to the network.

- **Hub**: the hub is the node that has the highest number of connections to other nodes. It represents the most important node of the network.

- **Eigenvalue centrality**: it is a measure that expresses how a node is accessible to other nodes.

- **Betweenness**: this measure tells how much a single node tends to be more central in the network, standing in between of other nodes' paths.

- **Nodal Centrality**: it expresses the importance of a single node depending on a measure given by degree, betweenness, eigenvalue or efficiency [61] [68].

7.8 Limits

Graph theory is not immune to limitations, given the difficulty of drawing specific conclusions, especially when a great multitude of factor is taken into account. The main discrepancy points are represented by:

- Differences given by the **chosen connectivity metric**;

- Differences given by the value of **thresholds**;

- Differences in **reference** locations recording;

- Definition of the **number of edges and nodes**;

- Bias given by **sample size**;

- **Population's characteristics**;

- **Brain conditions** of the subjects;

- Use of **weighted** or **unweighted** topologies.

All these factors added to the randomness of EEG's nature make it difficult to establish the most proper way of approaching graph theory. Literature is filled with different examples and experiments, trying to find the best combination of parameters, not without flaws. Unweighted networks seem to be capable of reducing the brain's network complexity by elimination weak connections. The proper number of electrodes and, therefore, of nodes is still matter of discussion, because of their effect on connectivity patterns. Even if large numbers seem to be preferred in literature, complex network derive from them, requiring advanced decomposition methods to draw conclusions. Regarding FC metrics, PL and CC have proven to be the most informative and most used because they present at best the network's characterization [62] [69].

Methods & Results

Chapter 8
Procedure

For this research, the EEG signal of several subjects affected by disorder of consciousness has been studied and analyzed with the goal of giving a causal and statistical interpretation to the severity of their disorder. Their EEG signals have been cleaned and processed, statistical measures were applied to them, and functional connectivity was used as the main tool of interdependency analysis.

The work was entirely carried out on a **Matlab code** (R2021a version), mainly using the **Signal Processing Toolbox**, in addition to external ones that will be discussed in their proper sections. A HP Laptop with the following specifications was used: **AMD Ryzen 7 4700U** processor with **Radeon Graphics**, running at **2.00 GHz**, **16.0 GB RAM**, **64 bits OS**. This information are noted as a reference of computational times and weights, for reproducibility sakes.

8.1 Data set

The data involved in this project have been provided by "**Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino**", to the "**Ontonix**" firm and to the "**Dipartimento di Scienze Applicate e Tecnologie**" of "**Politecnico di Torino**".

The dataset was composed of EEG signals registered from **54 subjects** in the years from 2011 to 2013. In detail, out of 54 subjects, **14** were **healthy subjects** whose signals were registered to create a reference, and **40** were **pathological patients** affected by consciousness disorders on different severity levels. The pathological patients were chosen as subjects who, due to severe cerebral lesions of traumatic or vascular origin, have suffered coma for a minimum time of 7 days and subsequent state of non-responsive vigilance or minimal conscious state. Patients of age inferior to 19 years and superior to 80, and patients whose EEG presented frequent epileptic anomalies were discarded, while the presence of sufficient clinical and anamnestic data of a patient was a necessary condition for their inclusion.

The EEG signals were, moreover, acquired in two conditions, with eyes open and eyes closed, and they were provided in two forms, raw and cut. The cut signals were cleaned in advance by removing extremely noisy or anomalous parts. All signals had the same duration of **256 seconds**, sampled at **256 Hz**.

The first choice that was made, was about the dataset to use: raw signals recorded with **closed eyes** were selected. **Raw time** series were preferred because the cut ones suffered from rigid removal of the noisy parts, which could have affected the analysis. Even if the raw signal might have included abnormal parts, proper pre-processing was applied. The condition regarding the eyes, instead, was chosen because with eyes open the EEGs could have been affected by any kind of visual stimulus and blinking artifact.

Since a certain number of patients suffered from severe DOC, the instruction of keeping the eyes open/closed was not always possible to be followed. In those case, the analysis was carried out anyways, pretending as if they followed the instruction, but keeping in mind that their signals might have been affected by said anomalies.

8.2 Channel selection

The EEG data of the patients were acquired using the **International 10-20 Electrode Placement System**. Because of the injuries of the patients or simply because of difficulties found in the placement of some electrodes, every subject results to have EEG signals acquired from different channels, and in a different number. To make the comparisons across patients more reliable and to ease the connectivity analysis, a subgroup of channels has been selected to carry out the work on. The channels subgroup was set by first identifying all the possible channels that have been used across the subject, and then non-informative channels and those that were not shared by all subjects were removed, as shown in *Table 4*. The starting data presented the signals in different orders depending on the patient, so a manual discarding was necessary. *Figure 25* was obtained using the *plot_topography.m* function, showing the final position of the 17 channels that satisfied the inclusion requirements. The whole study was accomplished on the data recorded on those channels, for all patients.

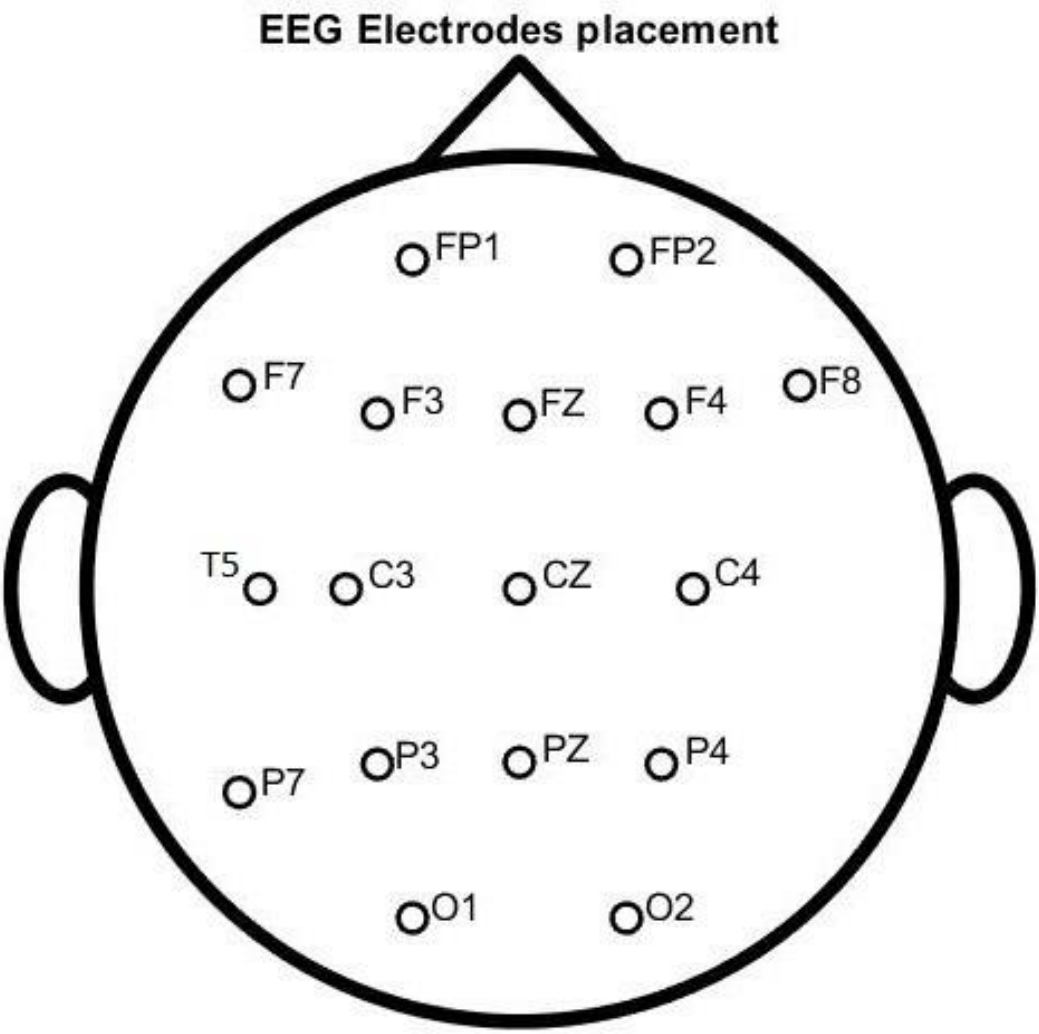


Figure 30. *Position of the chosen electrodes on the scalp.*

Table 4. Channels used for each subject for the purpose of eliminating the non-informative ones.

Patient	Fp1-RF	Fpz-RF	Fp2-RF	F7-RF	F3-RF	Fz-RF	F4-RF	F8-RF	A1-RF	15-RF	16-RF	T3-RF	C3-RF	Cz-RF	C4-RF	T4-RF	T5-RF	P3-RF	Pz-RF	P4-RF	T6-RF	O1-RF	O2-RF	TM-RF	MK-RF	AR-RF
1	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
2	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
3	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
4	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
5	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	
6	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
7	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
8	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X		X	X
9	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
10	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
11	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
12	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
13	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
14	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
15	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
16	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
17	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
18	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
19	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
20	X	X	X	X	X	X	X	X				X	X	X	X		X	X	X	X	X	X	X	X	X	
21	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
22	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
23	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
24	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
25	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
26	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
27	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
28	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
29	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
30	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X		X	X
31	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
32	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
33	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
34	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
35	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
36	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
37	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
38	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
39	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
40	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X		X	X	X	X	
41	X		X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
42	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
43	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
44	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
45	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
46	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
47	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
48	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
49	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
50	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
51	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
52	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
53	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
54	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Legend:

Electrode used: X

Electrode not used:

Channel discarded because not common

Channel discarded because not informative

8.3 Pre-processing

Once the starting point for each patient was established, the signals were test-plotted (*Figure 31*) with the *mostra_segnali.m* function and then subjected to pre-processing.

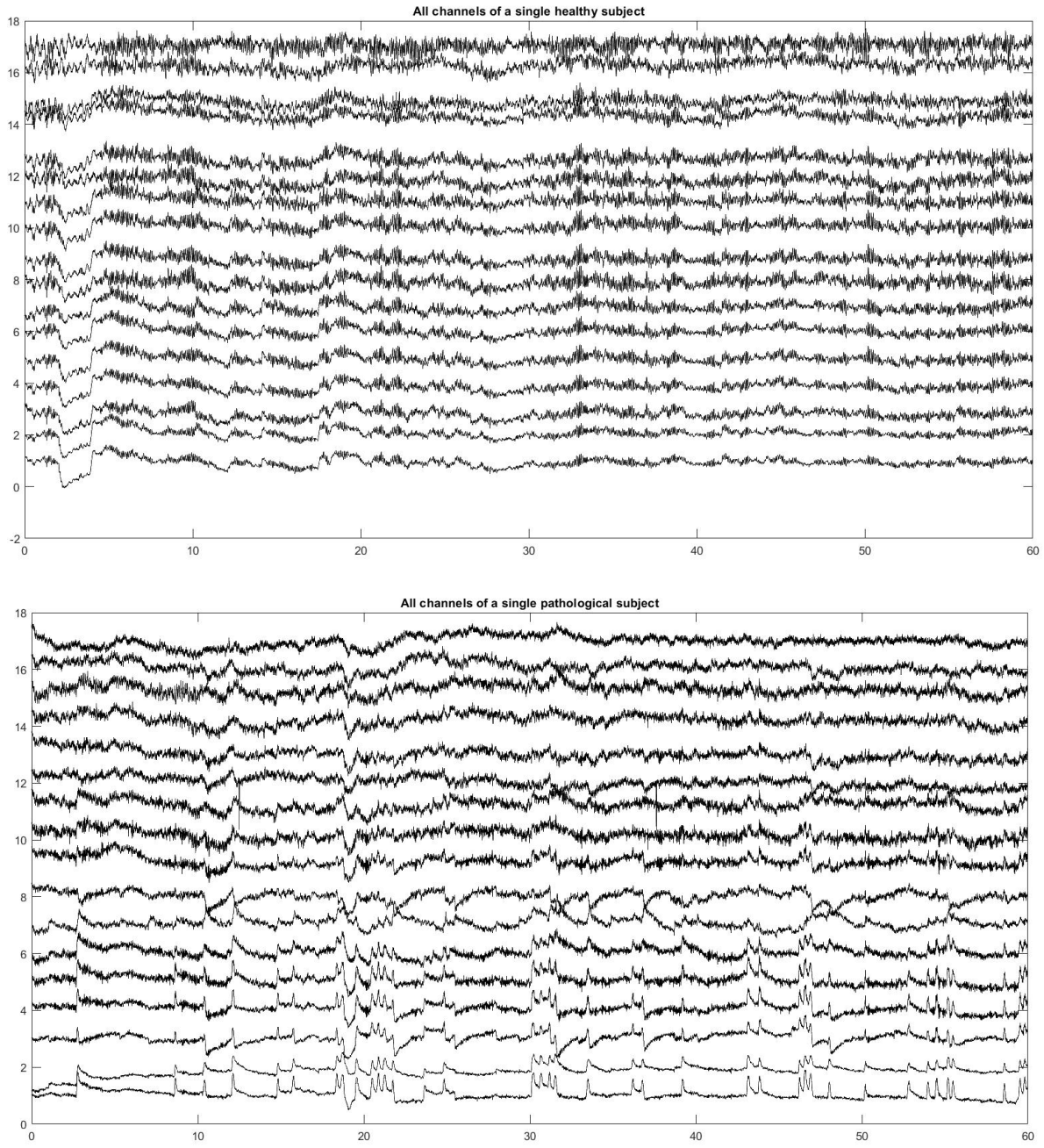


Figure 31. *Signals acquired from al 17 channels of a healthy subject (upper plot) and a pathological subject (lower plot).*

First, a **bandpass filter** was applied using the *BandPassFilter.m* function to identify and isolate the EEG frequency band, and to remove low frequency trends. The low and high cutoff frequencies were set respectively to **1 Hz** and **40 Hz**. Regarding the high cutoff frequency, this value was chosen because the Gamma band of the EEG was considered as non-informative in a study with this kind of patients, where the lower frequencies are predominant. Beta band was so considered as the last

informative band in the high frequencies, and its upper limit was kept wide. *Figure 28* shows the comparison of raw signals and filtered ones.

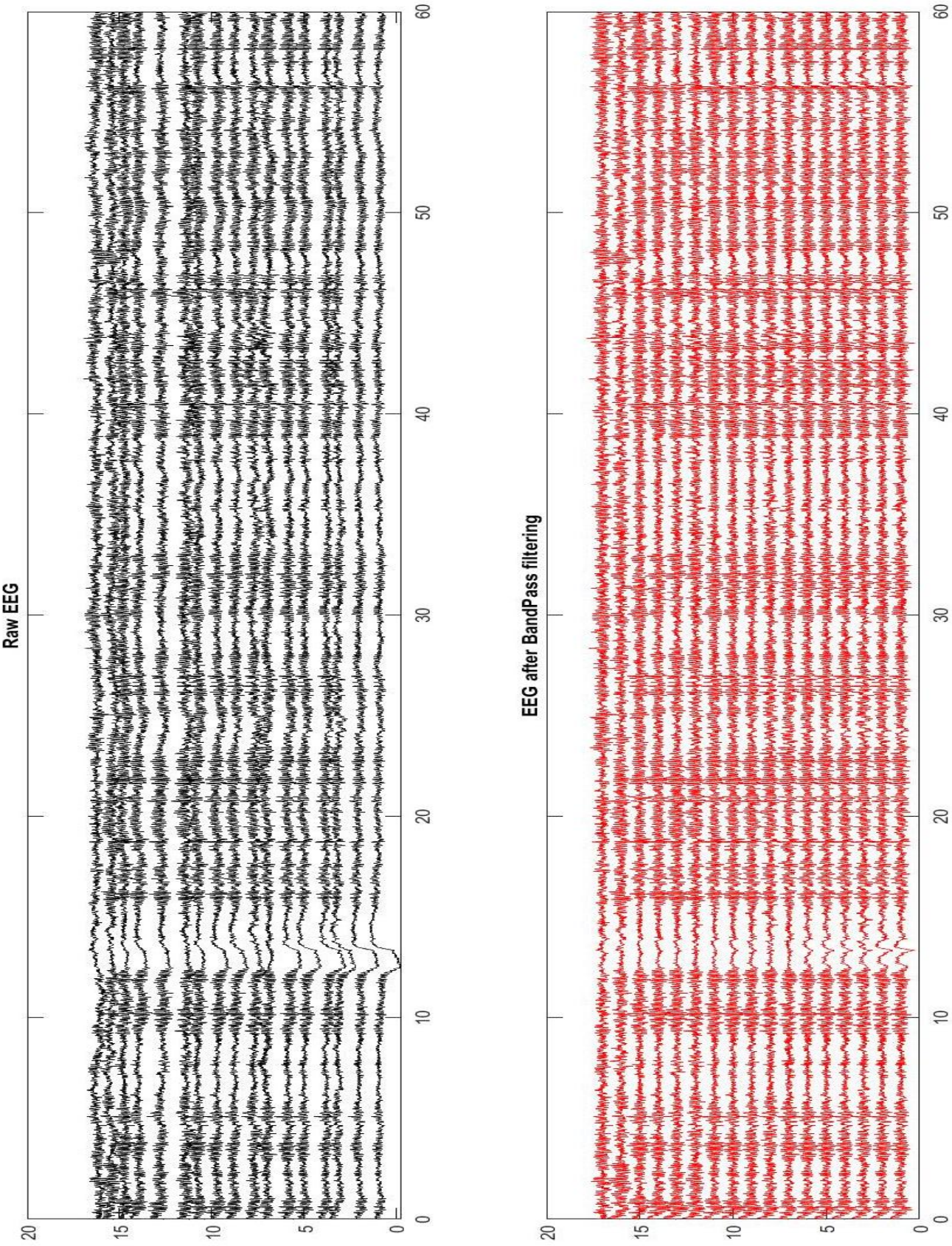


***Figure 32**. Comparison of Raw EEG and BandPass filtered EEG for all channels of a random subject.*

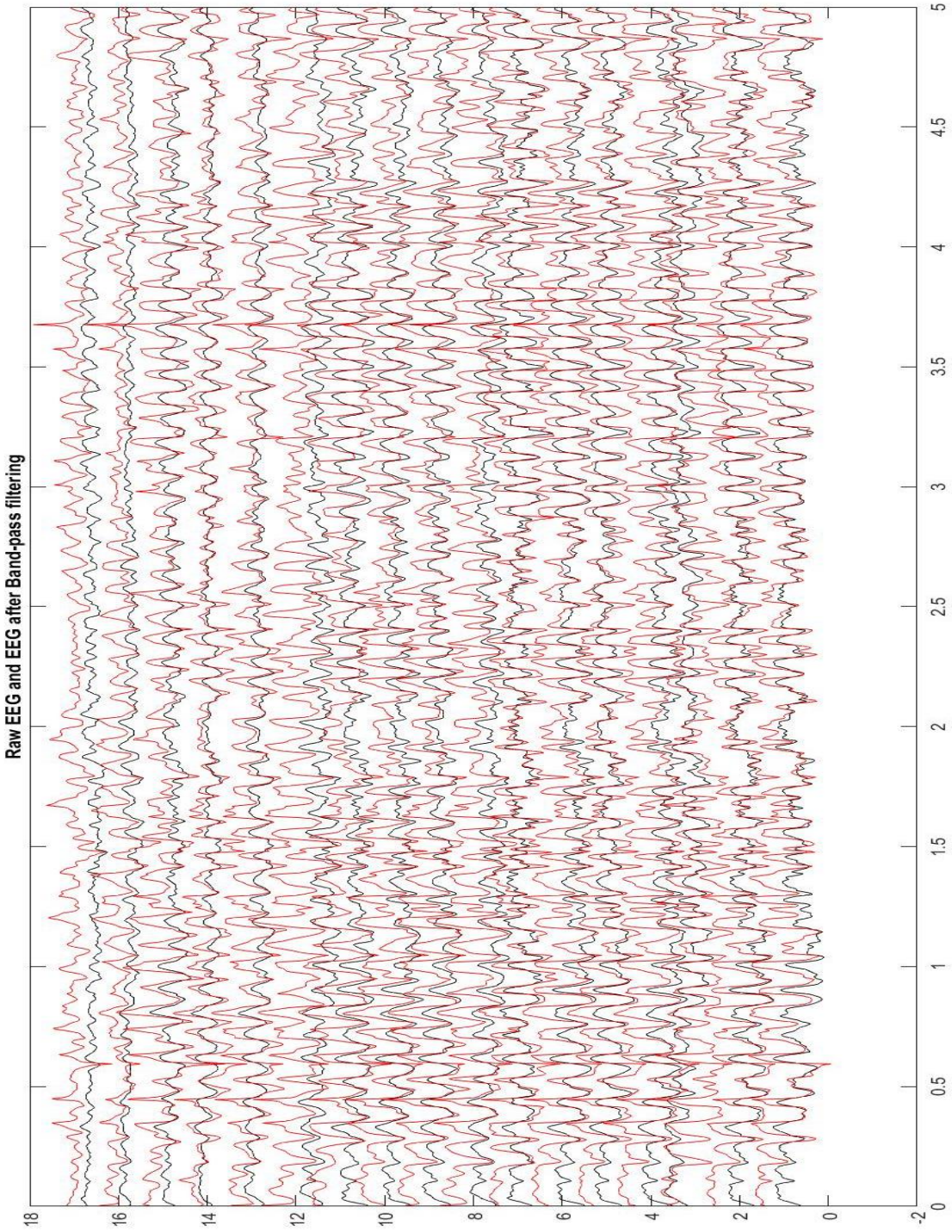


Figure 33. *Overlapping comparison of Raw EEG and BandPass filtered EEG for all channels of a random subject.*

Gomez's algorithm was employed to remove EOG and EMG artifacts [78]. It must be noted that EOG might not always be present because of the closed eyes condition, even though ocular movements might have been detected and corrected. EMG artifacts, instead, might have already been removed with the bandpass filter, as they are usually present in the high frequencies. The algorithm was implemented using the *autobss.m* function, with the following parameters:

```
% EOG artifact removing

    %Filter Parameters

    opt.wl = 200*fs;
    opt.ws = opt.wl;
    opt.bss_alg = 'sobi';
    opt.bss_opt = [];
    opt.crit_alg = 'eog_fd';
    opt.crit_opt.wl = 0.1*opt.wl;
    opt.crit_opt.ws = opt.crit_opt.wl;
    opt.crit_opt.method = 'sevcik_mean';
    opt.crit_opt.range = [];

    [single_output_eog] = autobss(patient_fil,opt);
    output_eog{k}=single_output_eog;


% EMG artifact removing

    %Filter parameters

    opt.wl = 3.12*fs;
    opt.ws = opt.wl;
    opt.bss_alg = 'bsscca';
    opt.bss_opt = [];
    opt.crit_alg = 'emg_psd';
    opt.crit_opt.femg = 15;

    opt.crit_opt.fs = fs;
    opt.crit_opt.ratio = 10;
    opt.crit_opt.estimator = [];
    opt.crit_opt.range = [];
    opt.crit_opt.NFFT = [];

    [single_output_emg] = autobss(patient_fil,opt);
    output_emg{k}=single_output_emg;

    [single_output] = autobss(output_eog{k},opt);
    output{k}=single_output;
```

Effects of Gomez's algorithm can be seen in *Figures 34, 35, 36, 37.*

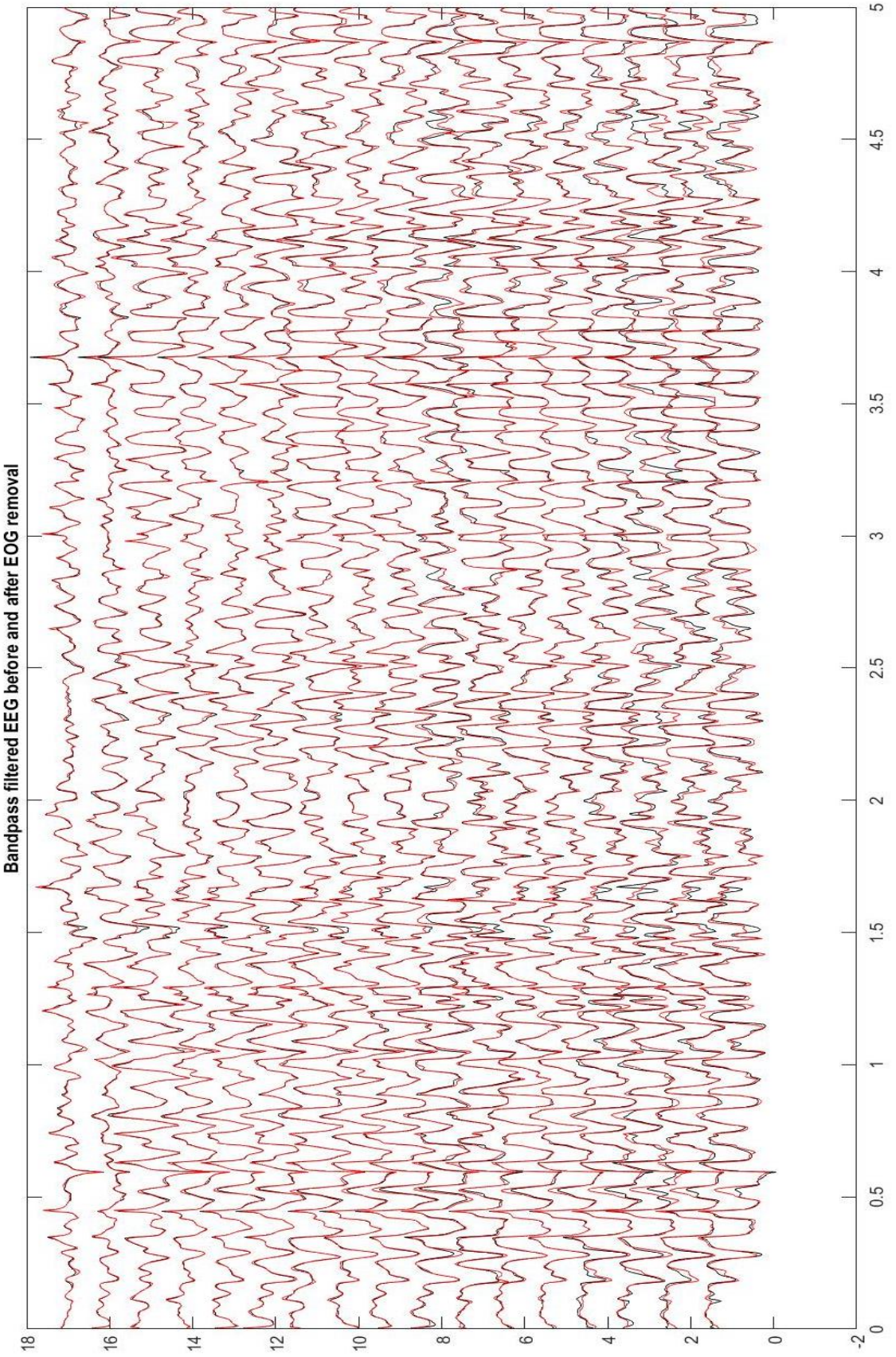


Figure 34. *Comparison of BandPass filtered EEG before and after EOG artifact removal, for all channels of a random subject.*

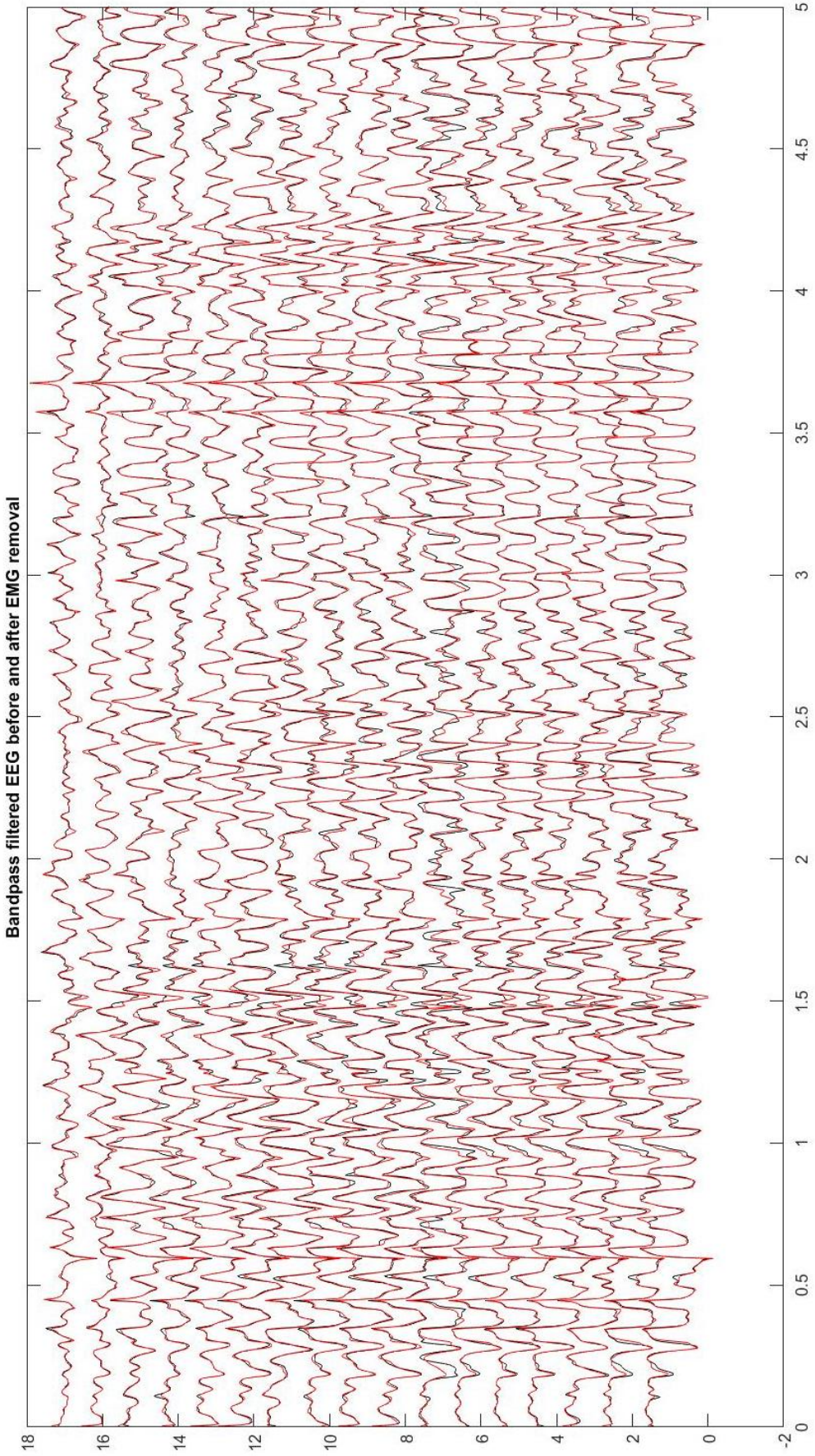


***Figure 35**. Comparison of BandPass filtered EEG before and after EMG artifact removal, for all channels of a random subject.*

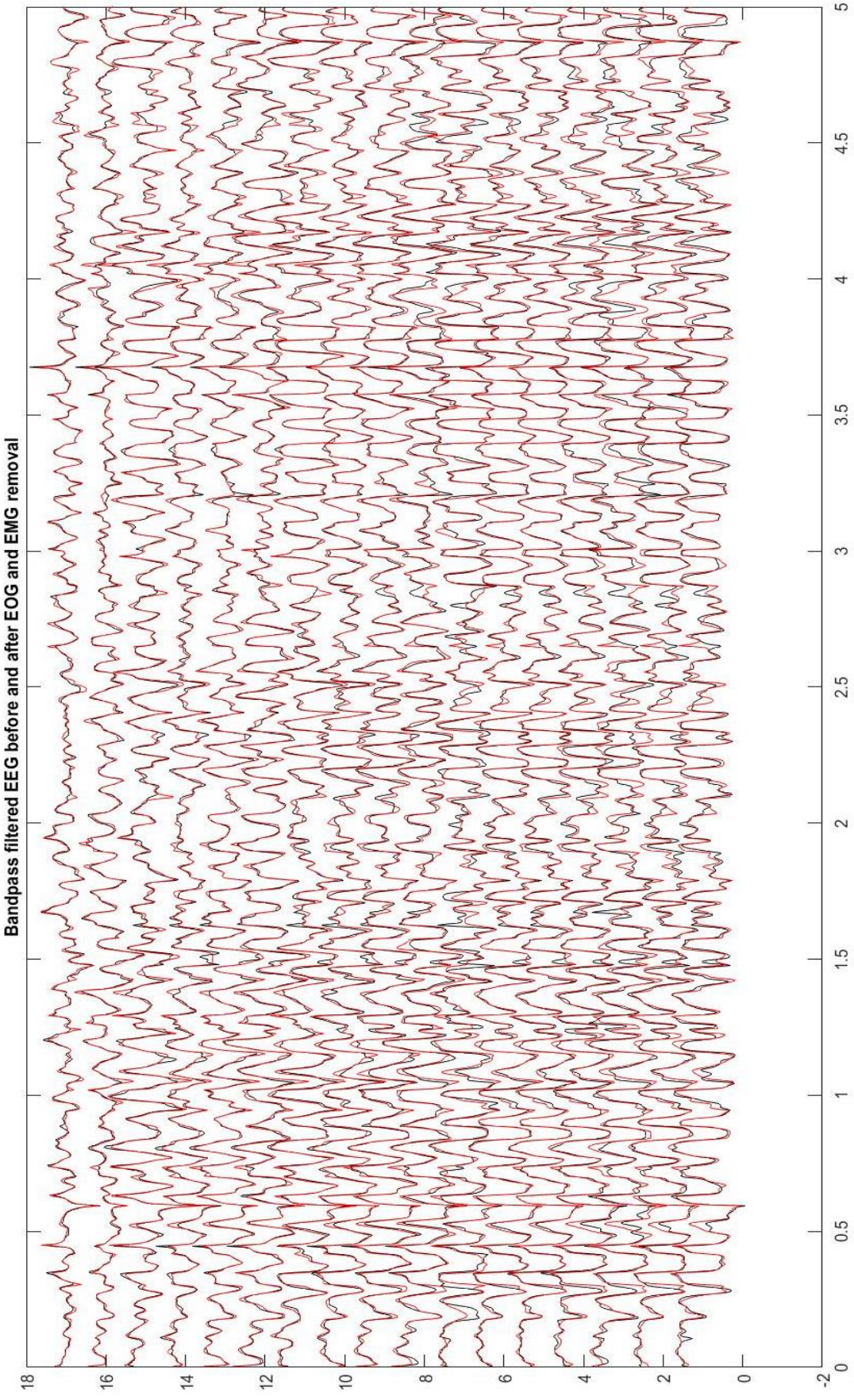


Figure 36. *Comparison of BandPass filtered EEG before and after both EOG and EMG artifact removal, for all channels of a random subject.*

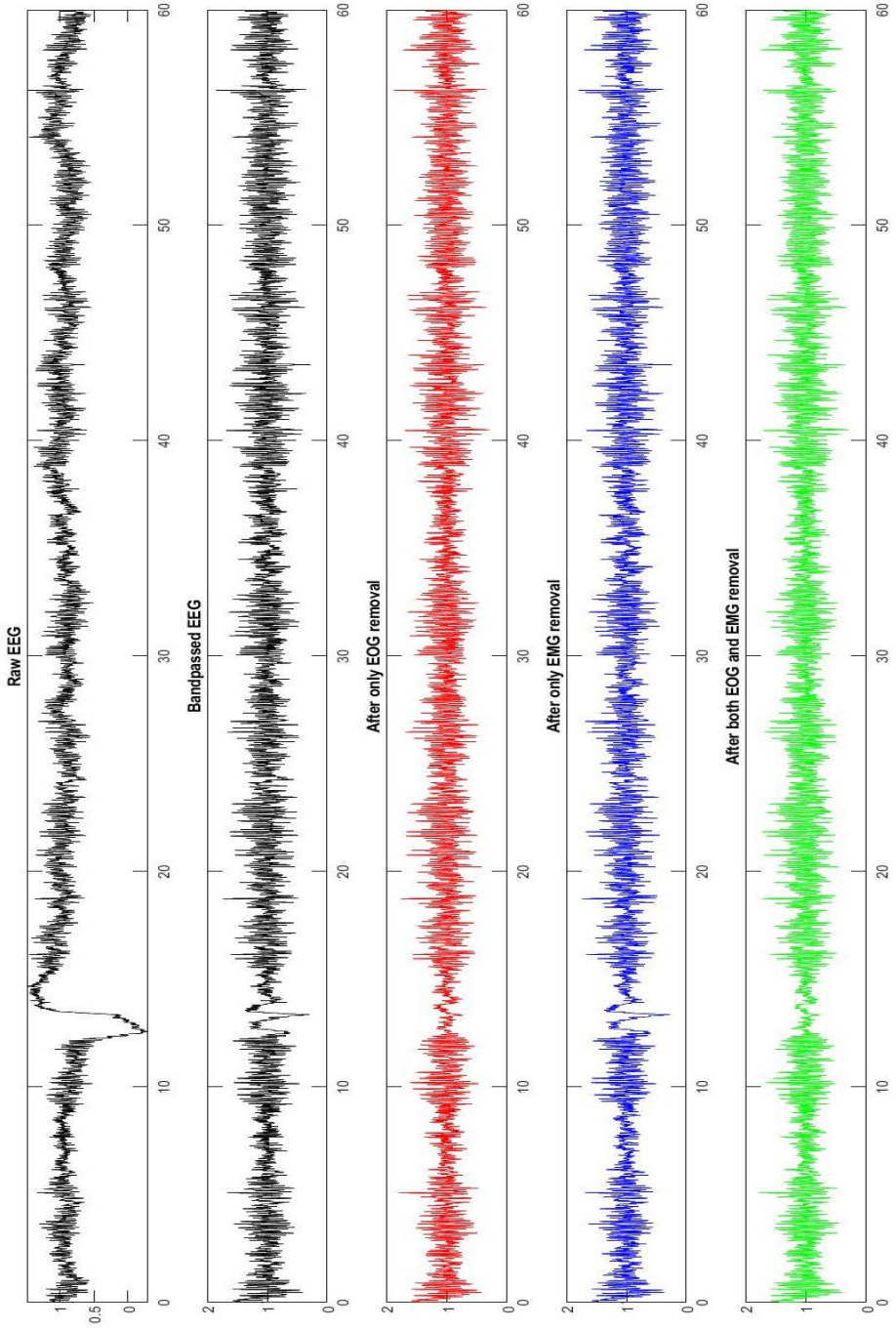


Figure 37. *Comparison of all the filtering cases: Raw EEG, band pass filtering, EOG artifact removal, EMG artifact removal and both EOG and EMG artifact removal on a single channel.*

8.4 Bands division

All the signals have been subsequently subjected to other bandpass filters to isolate all the frequency bands: **Delta** (1 - 3.9 Hz), **Theta** (4 – 7.9 Hz), **Alpha** (8 – 12.9 Hz) and **Beta** (13 – 40 Hz). Every statistical and connectivity measure that has been applied to the whole signal, from this point on has

been applied also to all the single frequency bands. *Figure 32* depicts all frequency band united with the whole signal.

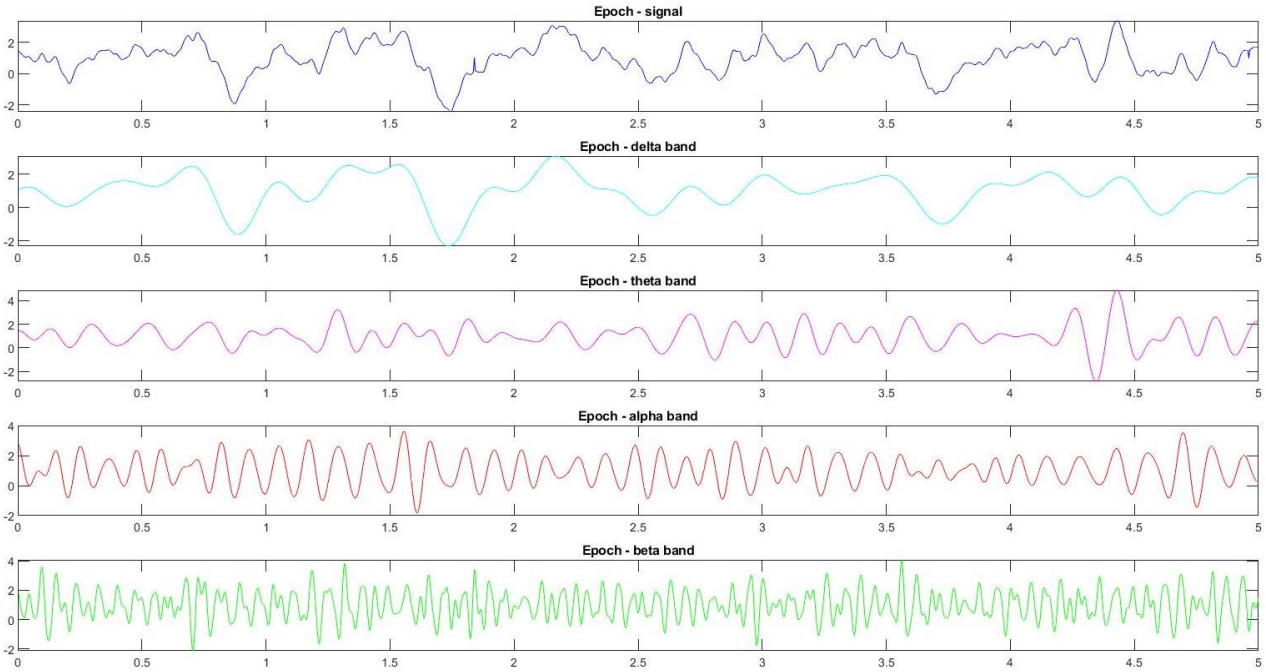


Figure 38. *Plot of all the frequency bands together with the whole signal, for one channel of a random patient.*

8.5 Epochs division

After the filters have been applied and the artifacts removed, all the signals were subdivided into non-overlapping epochs. The division in epochs was applied to the whole EEG signals and to the single frequency bands. After several tries with different epochs durations (1, 2, 5, 7), from 256 starting seconds for each signal, **51 epochs** lasting **5 seconds** each were constructed. The epochs division is necessary when using statistical and intercausal tools because, in order to get reliable results, the quasi-stationary condition needs to be satisfied. The duration of the epochs was decided as a compromise for getting the stationary condition and the need especially for FC measures to operate on epochs that were long enough to give consistent results. Lastly, the performances of the computer were also taken into consideration, to make the tests more efficient.

Every measure from this point on was calculated for all the Frequency bands, on the 51 epochs on all 17 channels of all 54 subjects.

8.6 Epochs parameters computation

Preliminary parameters were calculated for each epoch of each channel, for all the patients. When not specified, the parameter was computed manually:

- **Power Spectral Density** (PSD), using the following parameters:

```
T1 = epoc_len/2;
D = length(epochs{1,1}(1,1:T1))/2;  % Segment length
W = hamming(D);                     % Not rectangular window
O = fix(length(w)/2);               % Overlap expressed in samples
ris_teor = fs/D;                    % Theoretical Resolution
ris_app = ris_teor/4;               % Apparent Resolution
NFFT = 2^nextpow2(fs/ris_app);
```

- **Averaged Rectified Value** (ARV)

- **Root Mean Square** (RMS)

- **Mean Frequency** (MNF), using the *fmean.m* function

- **Median Frequency** (MDF), using the *fmedian.m* function

- **Shannon's Entropy** (ShEn), using the *ShanEn.m* function

These parameters were not considered for the final conclusions, but they were found anyways for completeness and to get an idea of their course.

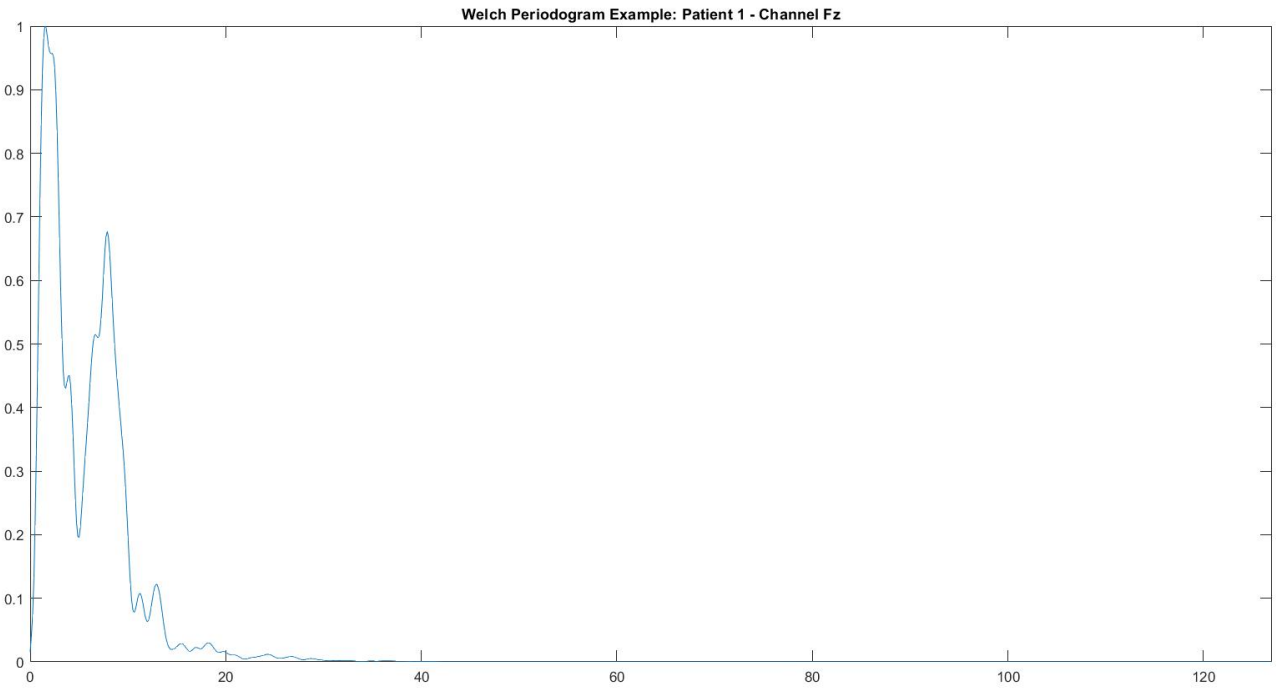


Figure 2. *PSD estimation example: Welch's Periodogram obtained from a singleepoch on a representative channel of a random subject.*

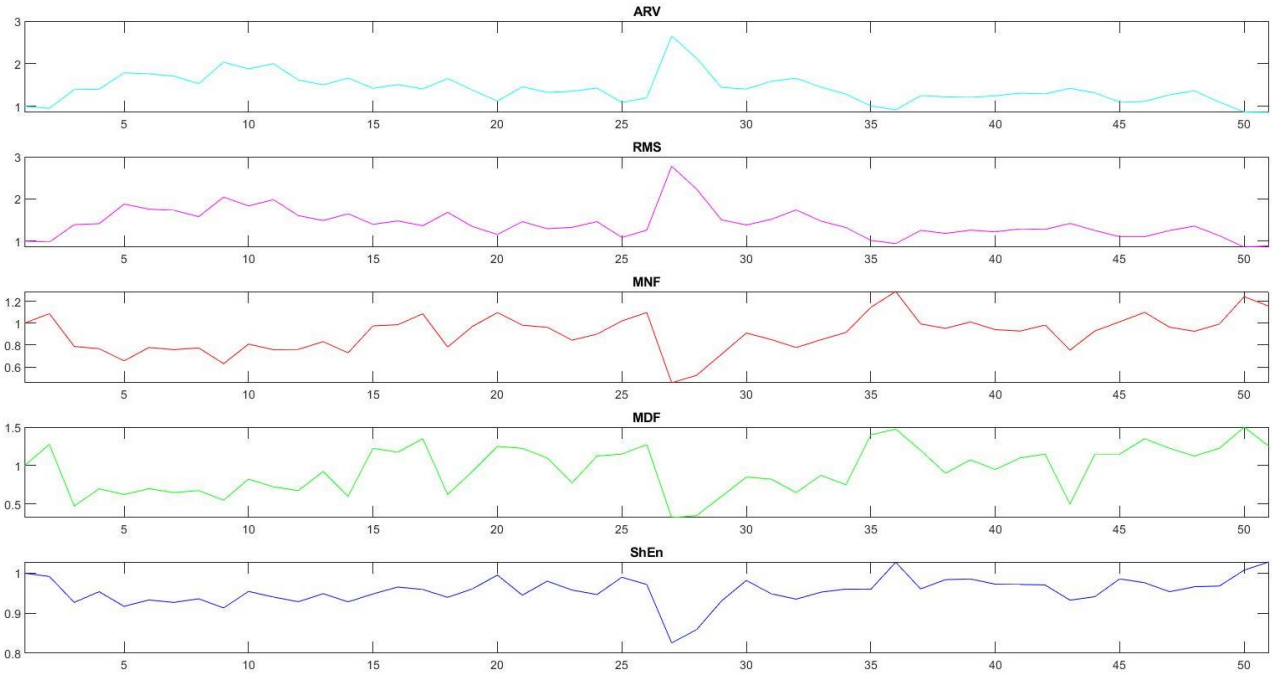


Figure 3. *ARV, RMS, MNF, MDF and ShEn calculated across epochs on a single channel of a random patient.*

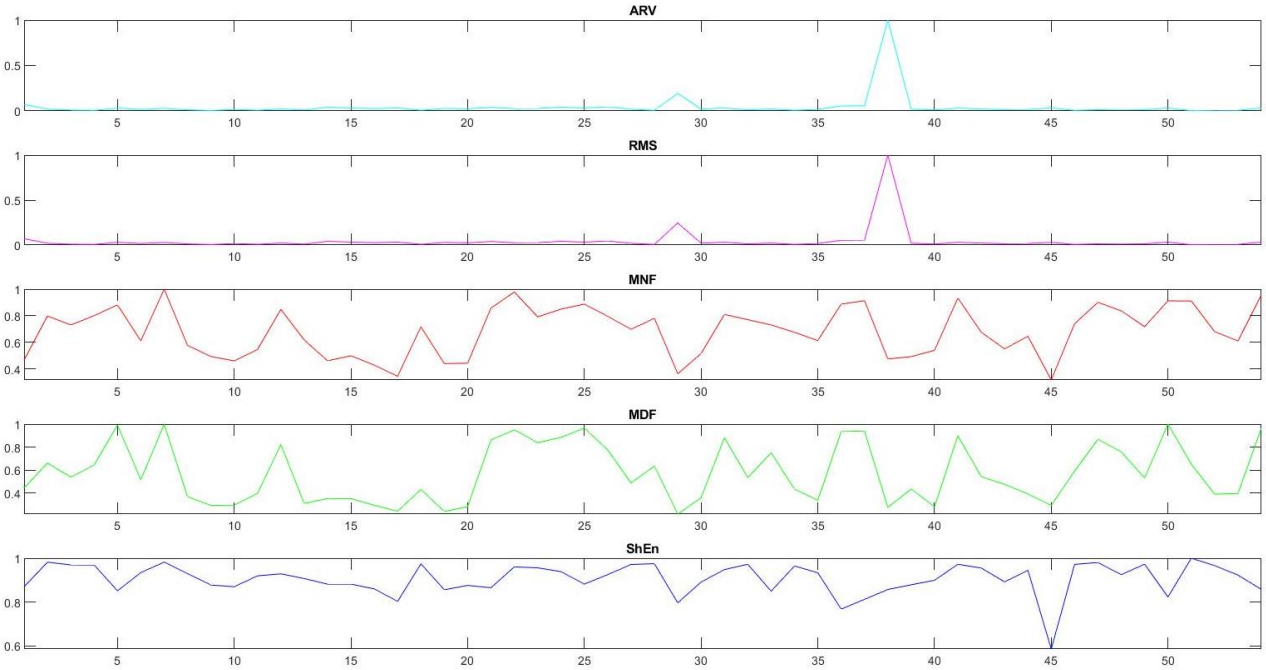


Figure 41*. Average ARV, RMS, MNF, MDF and ShEn on a single representative channel, across patients.*

8.7 Relative powers computation

The preliminary parameters were calculated on the whole frequency range, but, for each of the considered frequency bands, the powers relative to the single frequency bands were also computed. These measures are more meaningful because every EEG study concerning wakefulness starts by assessing the power relative to frequency bands more and less active in wake and sleep/unconscious states. For the most severe patient, the powers relative to the Beta band were expected to be low, as this frequency band is usually present during active thinking and thus wakefulness, with a predominance in the lower bands. On the other side, healthy subjects were expected to show homogeneous distribution of powers relative to the frequency bands.

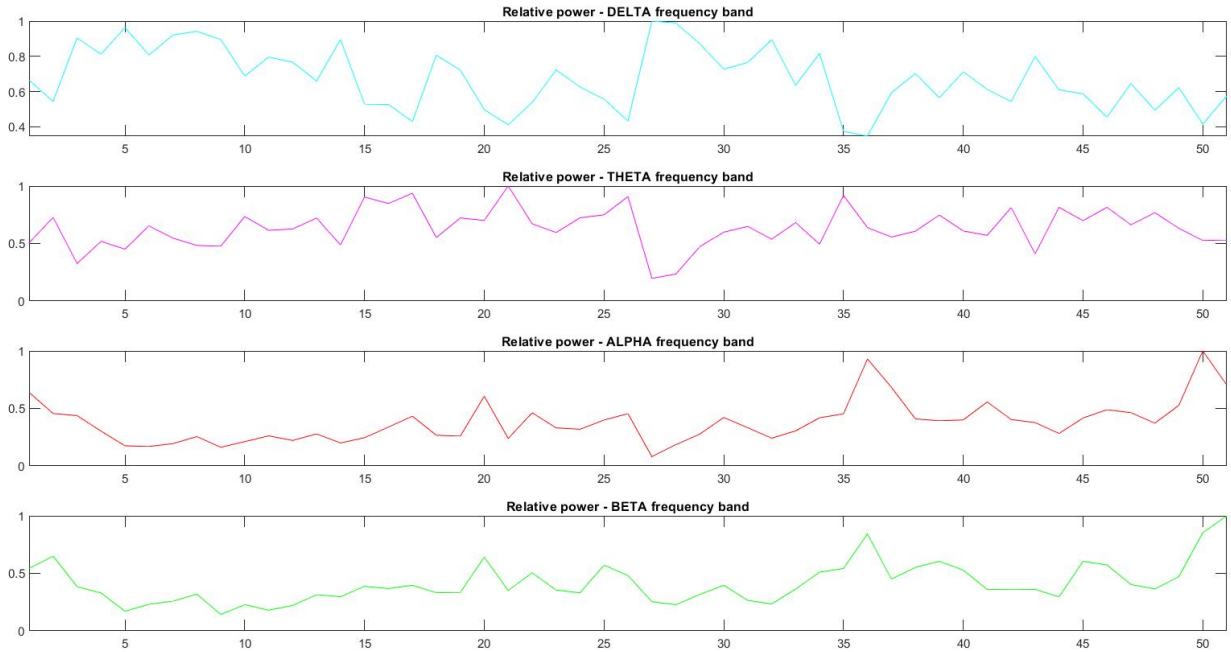


Figure 42. *Power relative to the different frequency bands across epochs, on a single representative channel of a random subject.*

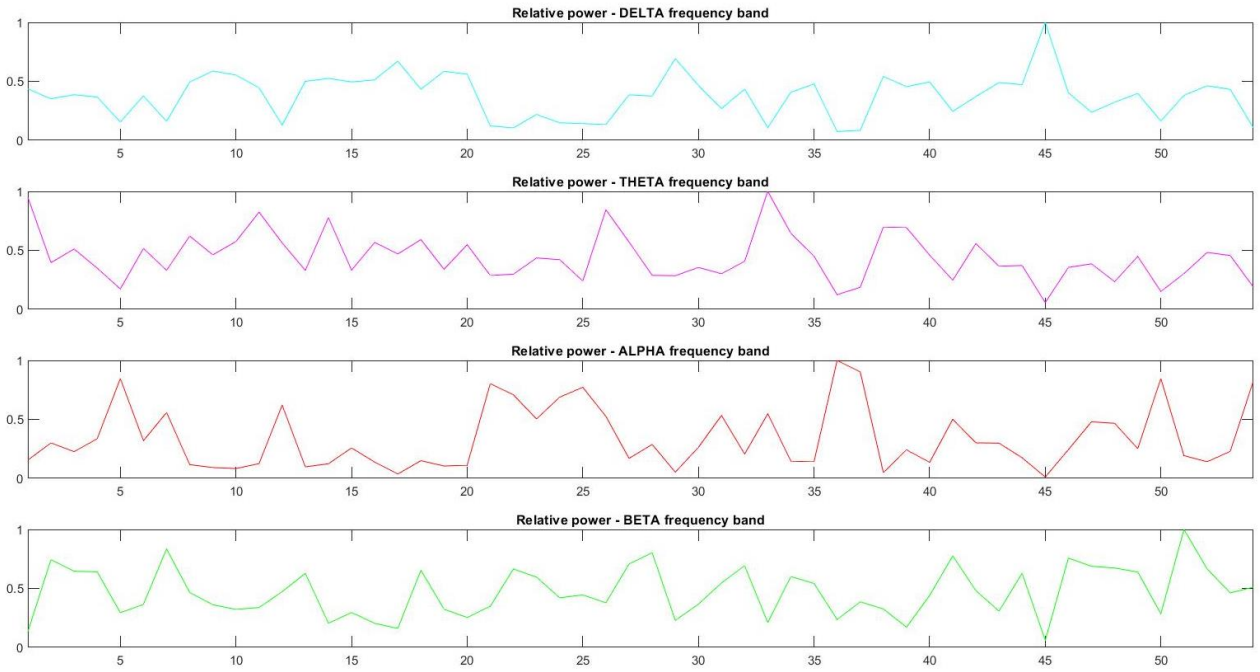


***Figure 43**. Average Power relative to the frequency bands across patients on a single representative channel.*

8.8 Approximate Entropy computation

ApEn was calculated for all the epochs of the signals and for each frequency band, in both its Original and Modified version, using respectively the *ApEn.m* and *ApEn_LM_opt.m* functions. In detail, a 4:1 resampling was made, passing from 256 Hz to 64 Hz, reducing the sample length of the epochs to overcome over-sampling and linearization of the signals. The parameters for the ApEn estimation were set as follows:

```
dim = 2;        % Embedding dimension
r = 0.2 ;       % Tolerance

p = 0.1;        % Percentage of the neighboring points
tau = 1;        % Delay
Theiler = 1;    % Theiler window
```

A Cross-Entropy estimation function was set to investigate inter-causalities across channels, but due to too much variety in the data and because of the heavy computation weights, this tool was discarded from the results.

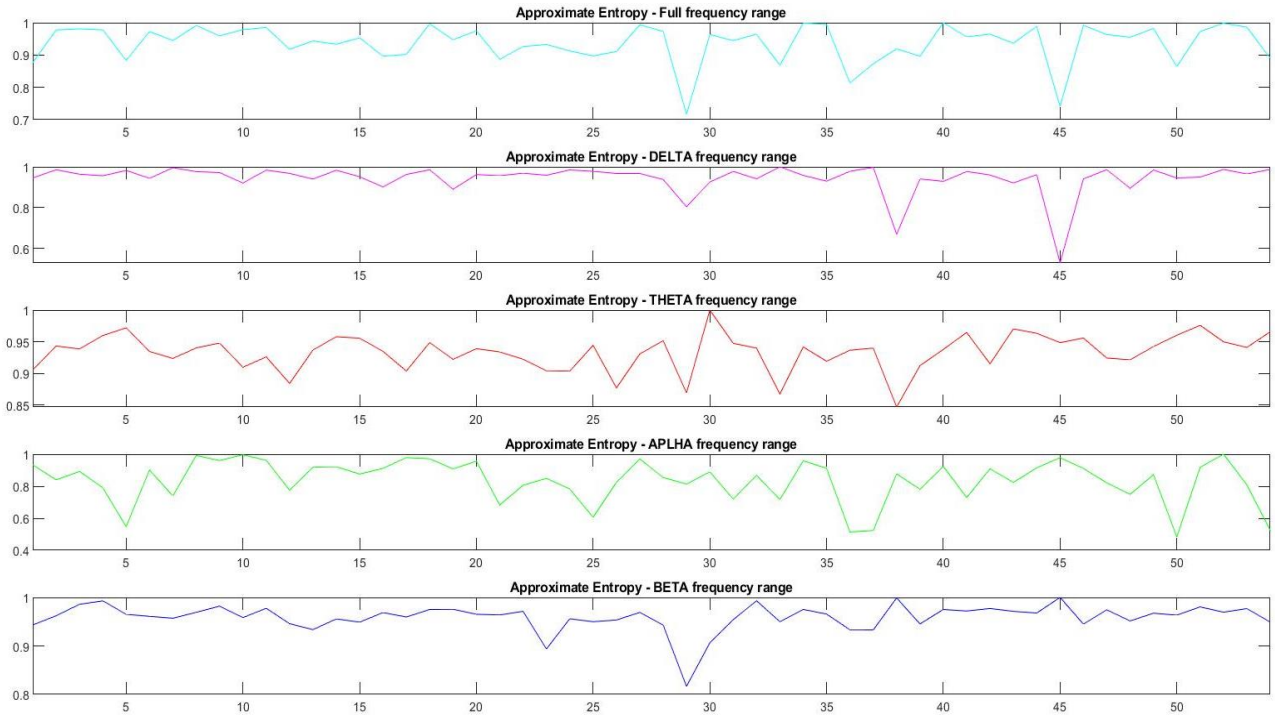


Figure 44. *Averaged Original Approximate Entropy on a representative channel across subjects.*

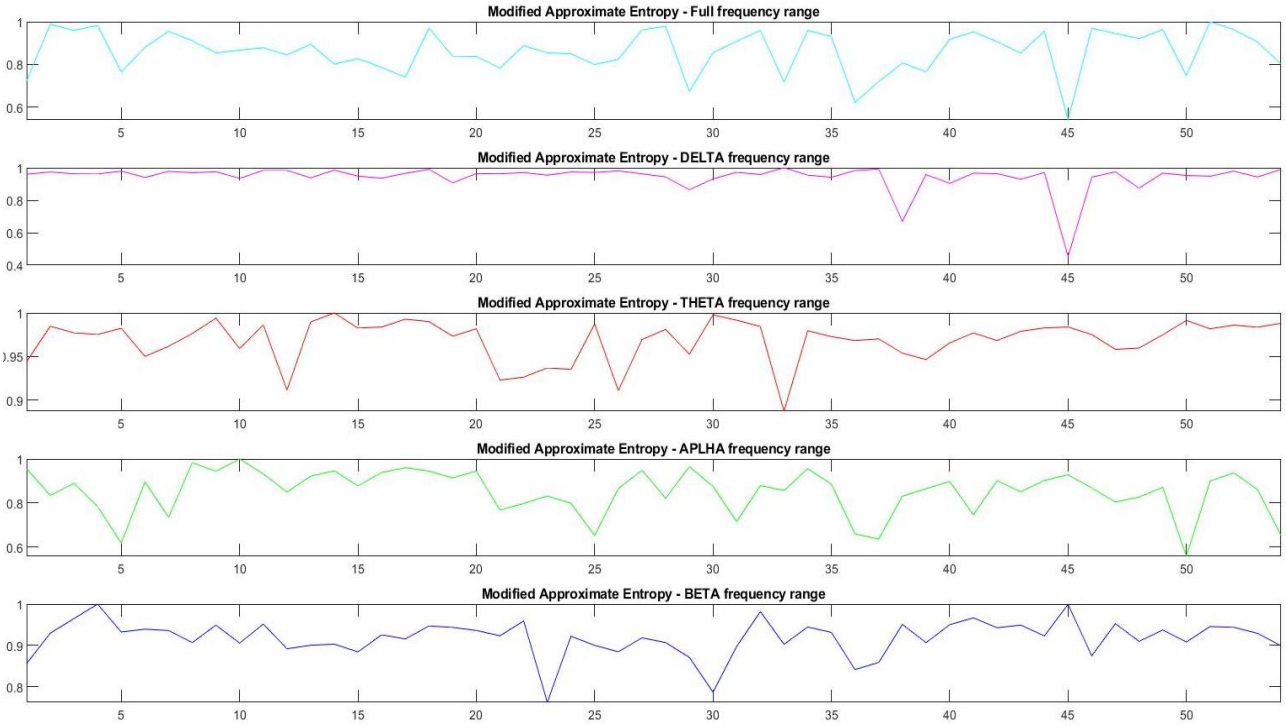


Figure 45*. Averaged Modified Approximate Entropy on a representative channel across subjects.*

8.9 Phase-Amplitude Coupling computation

The Cross-Frequency coupling was assessed through PAC computations. 4 different tools were used:

- **Canolty**'s formulation of Modulation Index

- **Ozkurt**'s formulation of Modulation Index

- **Tort**'s formulation of Modulation Index

- **Envelope-To-Signal** correlation

The first step consisted in finding the Amplitude and Phase of the signals. This was achieved with a Hilbert Transform. In particular, for PAC measures, the Phase is relative to the lower frequency band, while the Amplitude relates to the higher frequency band. For this work, two kinds of couplings were considered: **Delta-Alpha** and **Alpha-Beta**. These couplings were selected considering that in unconscious patients the Beta band is the weakest, and the Delta is the most present. This way, evident differences can be registered comparing healthy and pathological subjects.

In addition, since Tort's formulation evaluates the coupling on sections, the 360 degrees for the phase were divided into 18 bins.

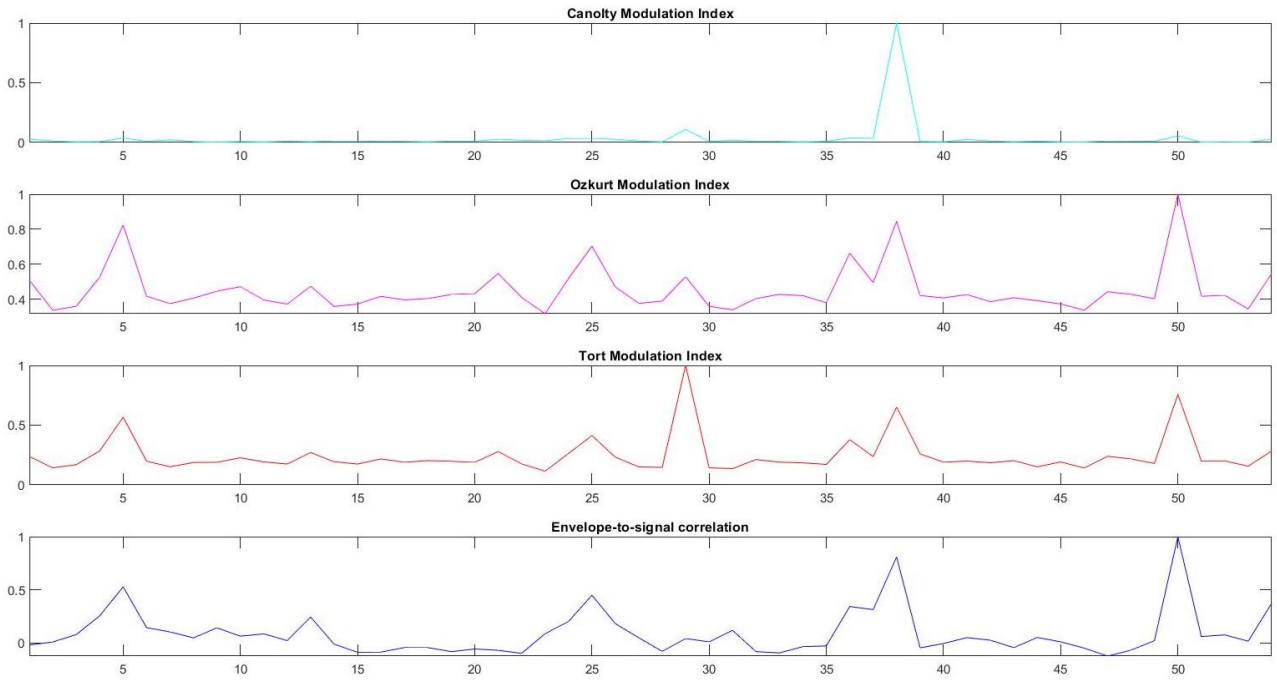


***Figure 46**. PAC measures on a representative channel across subjects.*

8.10 Functional connectivity

FC analysis was carried out with the aid of the **Brain Connectivity Toolbox** (BCT), Version of 2019. This toolbox was developed in 2009 by M. Rubinov and O. Sporns as an open-source Matlab toolbox. In the following years it was then ported to programming languages, included into several projects, and updated to improve network analyses. The main benefits deriving from BCT's usage lay in the possibility to have easily accessible functions, available in both directed and undirected, or weighted and unweighted variants, permitting manipulation and customization of the networks. For this study, BCT was mainly used for representing adjacency matrices and brain networks, matrix thresholding and binarization, and FC indexes calculation [79].

8.10.1 Connectivity measures computation

The first step in applying FC was computing different correlation metrics. A number of them have been chosen to compensate for each other's limitations. Moreover, since different connectivity metrics have different characteristics in terms of domain, linearity, sensitiveness and direction of analysis, studying more than one measure resulted to bring to more comparable and reliable results.

All the correlation measures were computed for each epoch of the signals, for every combination of paired channels, for each frequency band of each subject.

The connectivity measures that have been employed are the following:

- **Mutual Information**: MI was obtained using the *kernelmi.m* function, which automatically sets a kernel from which the marginal and joint probabilities are computed. It was normalized for comparisons between channel pairs;

- **Covariance**: manually obtained computing the covariance matrix. It was normalized;

- **Pearson's Correlation Coefficient**: directly obtained starting from the Covariance value. It was also normalized;

- **Cross-correlation**: manually computed by looking for the maximum value of the cross-correlation between the signals pair;

- **Phase-Locking Value**: calculated using the *PLV.m* function, which gives the measure both in its original form and after a phase smoothing;

- **Phase-Lag Index**: manually computed starting from the phase of the signals obtained with a Hilbert transform. The values were normalized.

8.10.2 Adjacency matrices creation and representation

The Adjacency (or Connectivity) matrices were constructed:

- for each correlation metric,
- for each frequency band,

- for each epoch of signals
- for all subjects.

Every connectivity matrix is a **17x17 matrix** because 17 are the channels/nodes involved, and every element composing them is given by the correlation measure (edge) between the corresponding channels pair. The adjacency matrices result to be undirected because of the correlation measures that were used.

Corresponding to every adjacency matrix, a representation of the brain's network was made using the *adjacency_plot_und.m* function of the BCT.

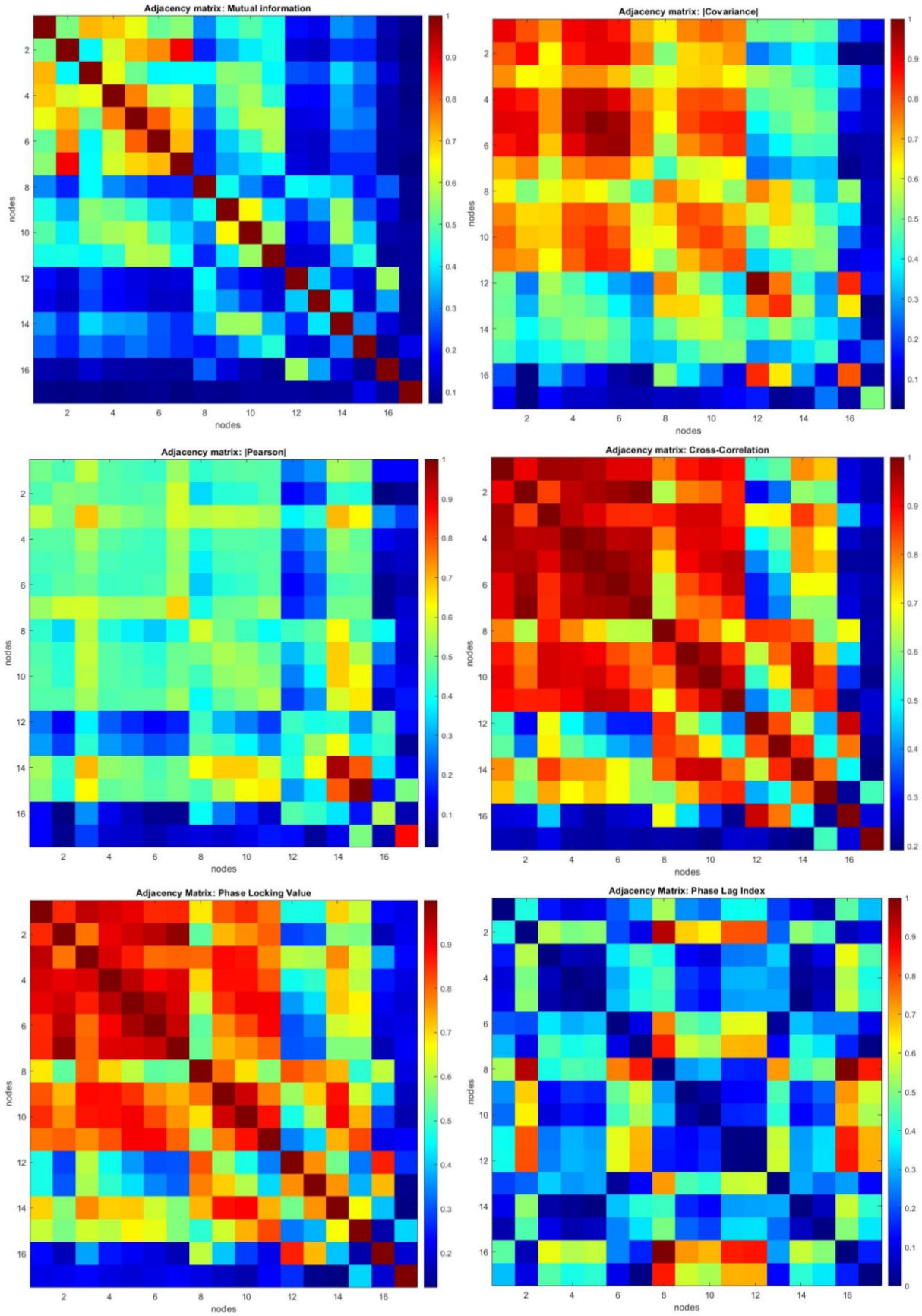


Figure 47*. Adjacency matrices examples for each of the employed correlation measures (in order: Mutual Information, Covariance, Pearson's Correlation Coefficient, Cross-Correlation, Phase-Locking Value and Phase Lag Index), obtained on a random epoch, on EEG's whole frequency range, from a random subject.*

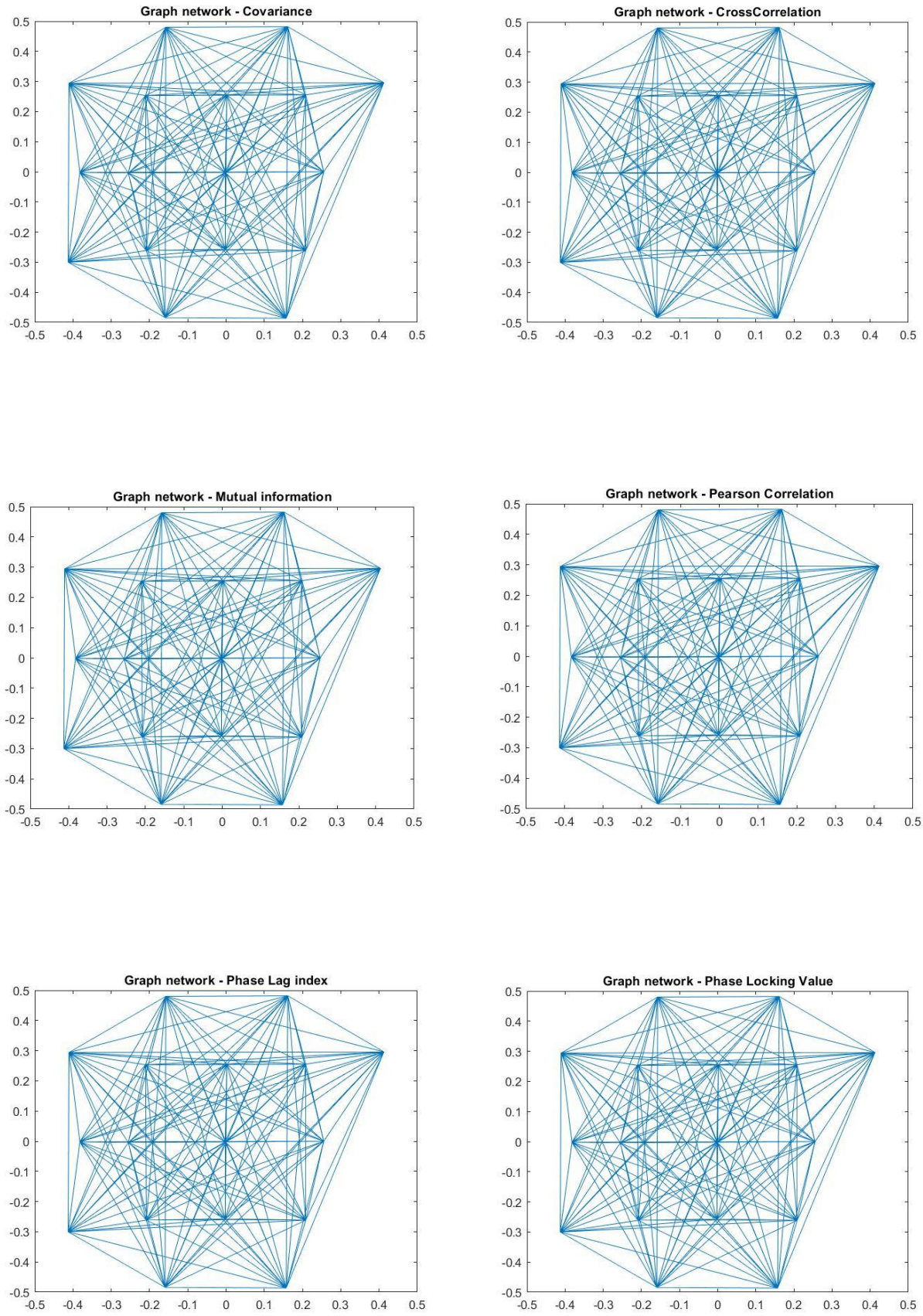


Figure 48. *Brain/Graph's Network representation examples with their Nodes and Edges positioned according to the electrode coordinates on the scalp. These Graphs appear equal to each other because every considered channel has a connection to the others. The difference lays in the weights of each connection that is visible, instead, in the Connectivity Matrices.*

8.10.3 Adjacency Matrices Thresholding

A threshold was applied to all the adjacency matrices to eliminate weak connections and, as a consequence, false positives. Values above the threshold were left unchanged. The threshold was set to **0.3**, the same for every correlation, considering that these were normalized with values between 0 and 1. Since thresholding is a very heated topic of discussion in literature, finding the appropriate value is not easy. Its value was chosen after attempting different thresholds (0.2, 0.25, 0.3, 0.4), and relying on results found in the literature relative to similar studies. The thresholding was obtained using the *threshold_absolute.m* function of the BCT. This function, in addition to the thresholding, clear the main diagonal of the matrices, to remove the connection of a node with itself.

Figure 49 and *Figure 50* show the connectivity matrices and the graphs that derive from the thresholding.

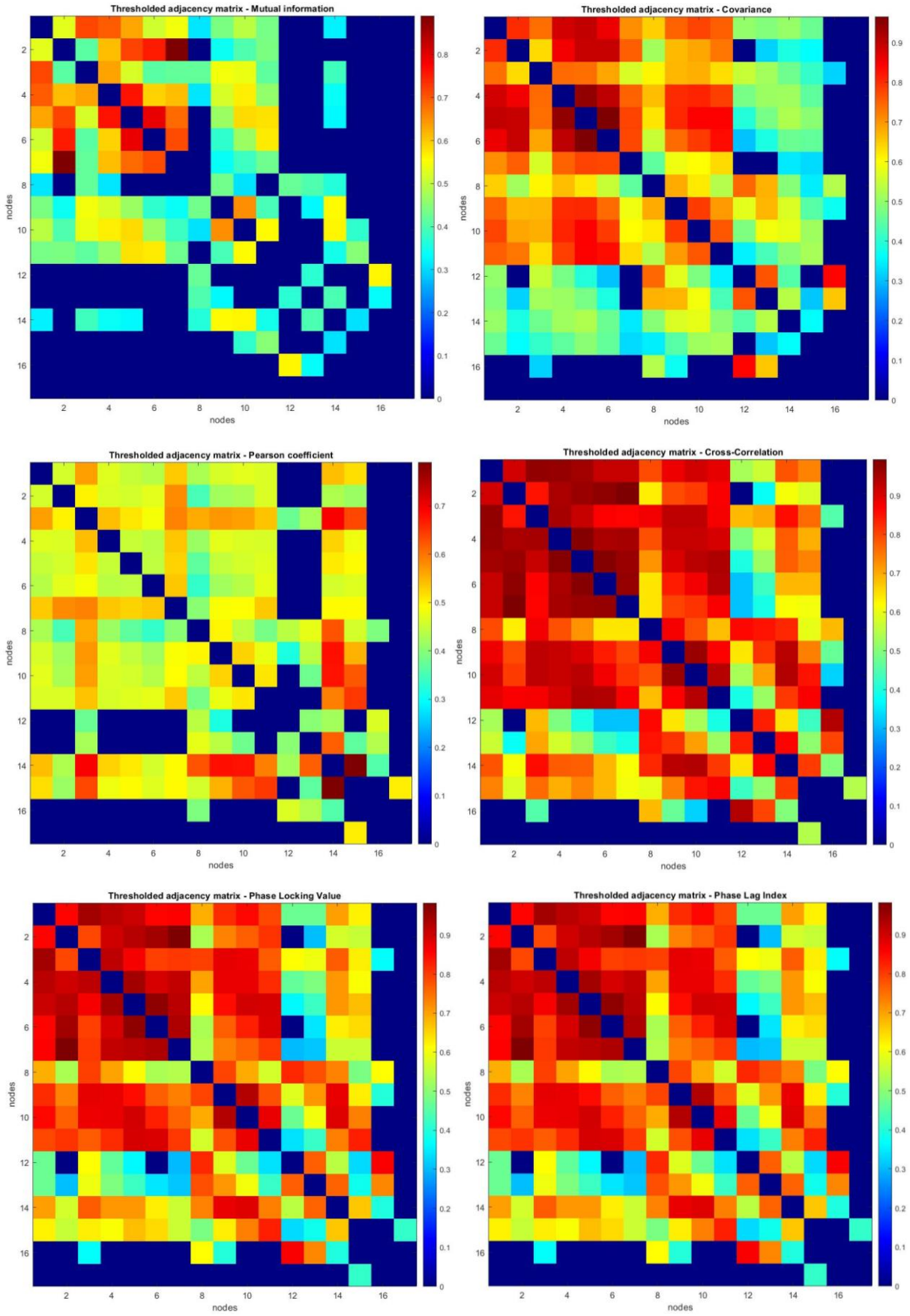
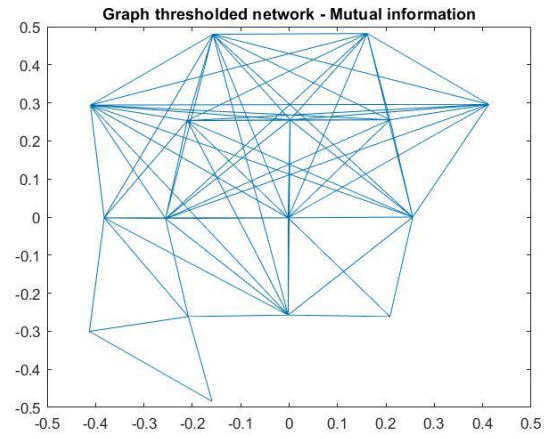
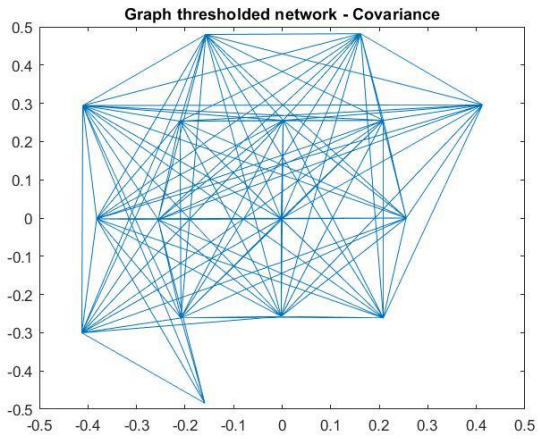
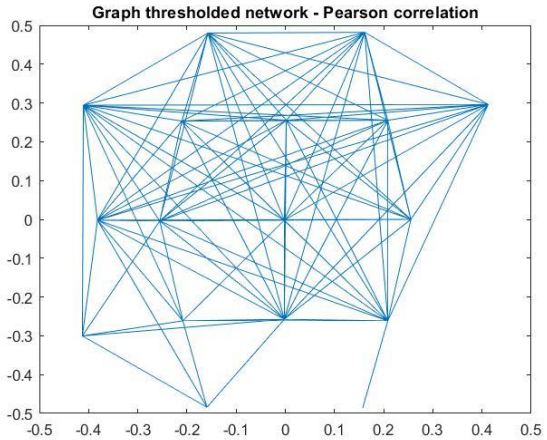
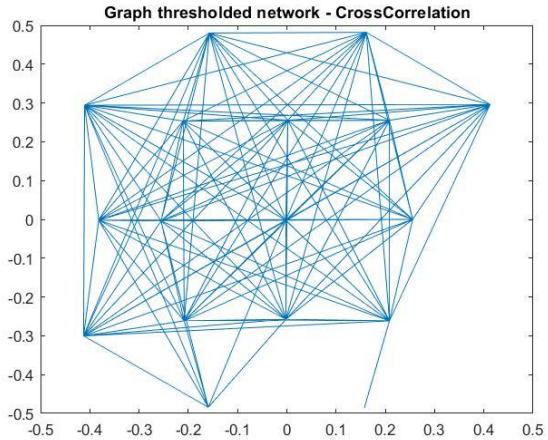


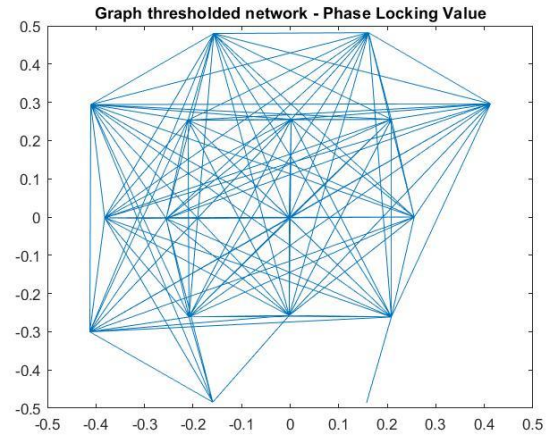
Figure 49. *Thresholded Adjacency matrices examples for each of the employed correlation measures (in order: Mutual Information, Covariance, Pearson's Correlation Coefficient, Cross-Correlation, Phase-Locking Value and Phase Lag Index), obtained on a random epoch, on EEG's whole frequency range, from a random subject.*











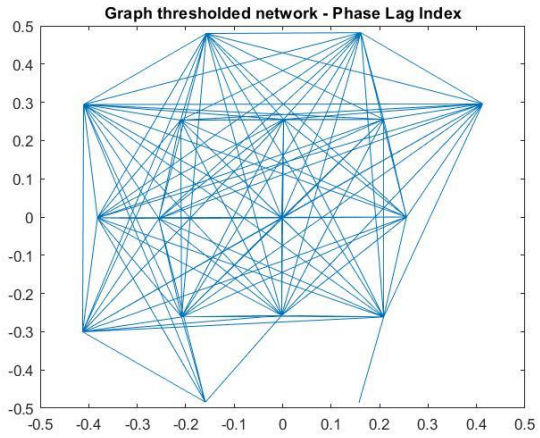


***Figure 50**. Thresholded Brain/Graph's Network representation examples with their Nodes and Edges positioned according to the electrode coordinates on the scalp. These Graphs appear different from each other because weak connections have been eliminated, and they are different from one connectivity measure to another.*

8.10.4 Adjacency Matrices Binarization

Separately from the previous thresholding, the original adjacency matrices found in *Section 8.10.2* were submitted to another kind of threshold. This time the matrices were binarized according to the threshold, as the values below were put to 0, while the connections above were put to 1. The binarization of the matrices was employed to take into consideration only strong connections, independently from their weight. It was manually obtained, but the binarization threshold value was set differently according to the connectivity measure. In particular, **0.5** was employed for Mutual Information and Pearson's Correlation Coefficient, **0.7** for Covariance, and **0.8** for Cross-Correlation, Phase-Locking Value and Phase Lag Index. These values derive from a trial-and-error approach, trying different values found in literature. The values are different from each other because in order to narrow the field of the range of connections, for some correlation coefficients higher threshold values resulted necessary. Moreover, the binarization thresholds are all higher than the value of the thresholding carried out in *Section 8.10.3* because in this case only strong connections are preserved for the following analysis.

Figure 51 and *Figure 52* show the connectivity matrices and the graphs that derive from the binarization.

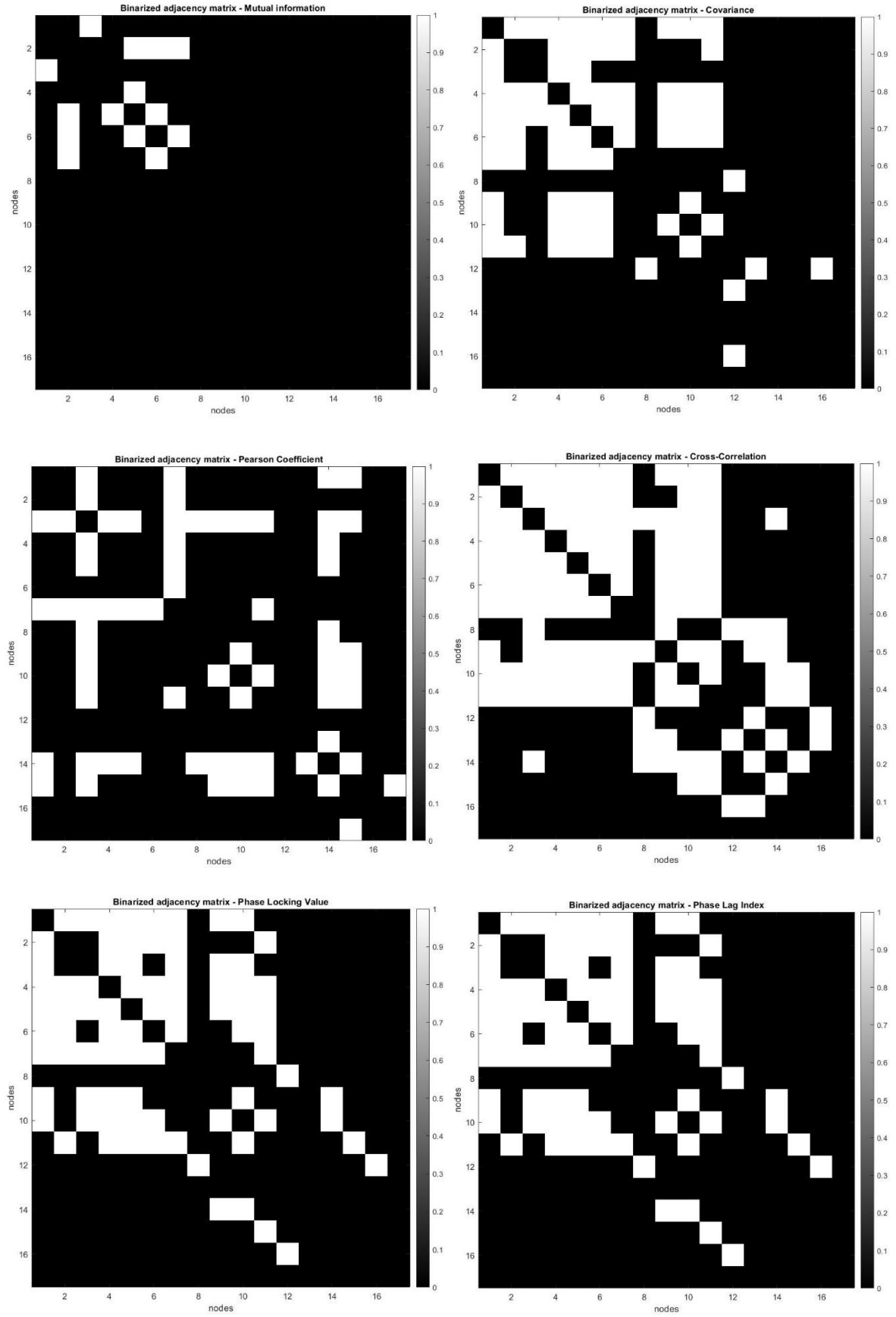


Figure 4. *Binarized Adjacency matrices examples for each of the employed correlation measures (in order: Mutual Information, Covariance, Pearson's Correlation Coefficient, Cross-Correlation, Phase-Locking Value and Phase Lag Index), obtained on a random epoch, on EEG's whole frequency range, from a random subject.*

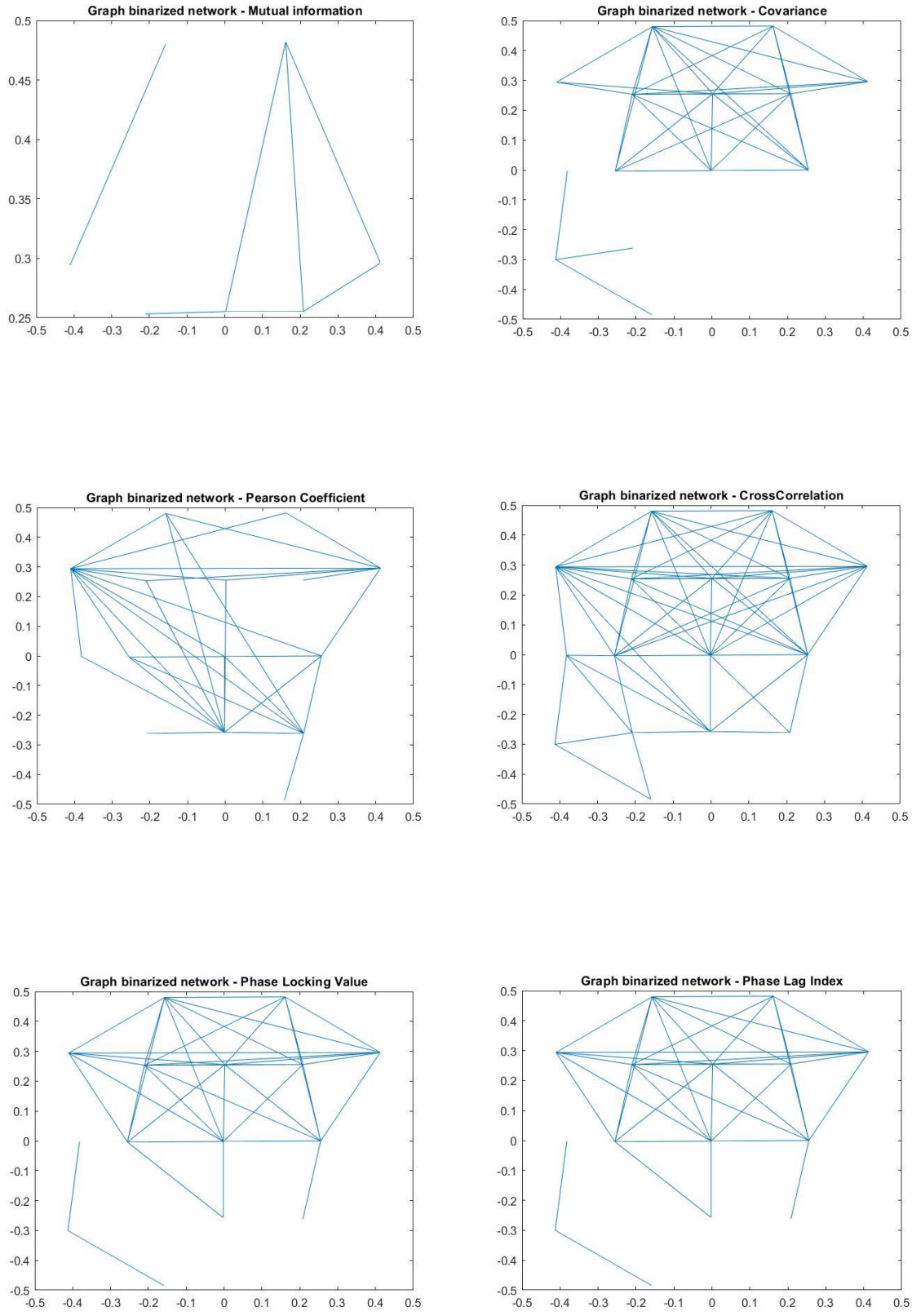


Figure 52. *Binarized Brain/Graph's Network representation examples with their Nodes and Edges positioned according to the electrode coordinates on the scalp. These Graphs appear different from each other because weak connections have been eliminated, and they are different from one connectivity measure to another.*

8.10.5 Functional Connectivity Indexes

The BCT was mainly used to calculate global and local FC indexes. The functions were separately applied to the weighted Thresholded and unweighted Binarized Adjacency Matrices, as always corresponding to every epoch, of every channel, of every patient, on all the frequency ranges.

The calculated **Global Indexes** were:

- **Characteristic Path Length**, obtained using the *distance_bin.m* and *distance_wei.m* function, and the *charpath.m* function;
- **Transitivity**, using the *transitivity_bu.m* and *transitivity_wu.m* functions;
- **Global Efficiency**, as a further output of the *charpath.m* function.

The **Local Indexes** were:

- **Clustering Coefficient**, obtained using the *clustering_coef_bu.m* and *clustering_coef_wu.m* function*;*
- **Local Efficiency**, using the *efficiency_bin.m* and *efficiency_wei.m* functions*;*
- **Nodal eccentricity**, as a further output of the charpath.m function;
- **Small-worldness**, using the values of *Clustering Coefficient and Characteristic Path Length;*
- **Degree**, using the *degree_und.m function;*
- **Betweenness**, using the *betweenness_bin.m* and *betweenness_wei.m* function*s;*
- **Eigenvector Centrality**, using the *eigenvector_centrality_und.m* function;
- **Assortativity**, using the *assortativity_bin.m* and *assortativity_wei.m* functions*;*
- **Reachability**, using the *breadthdist.m* function, only compatible to binarized matrices.

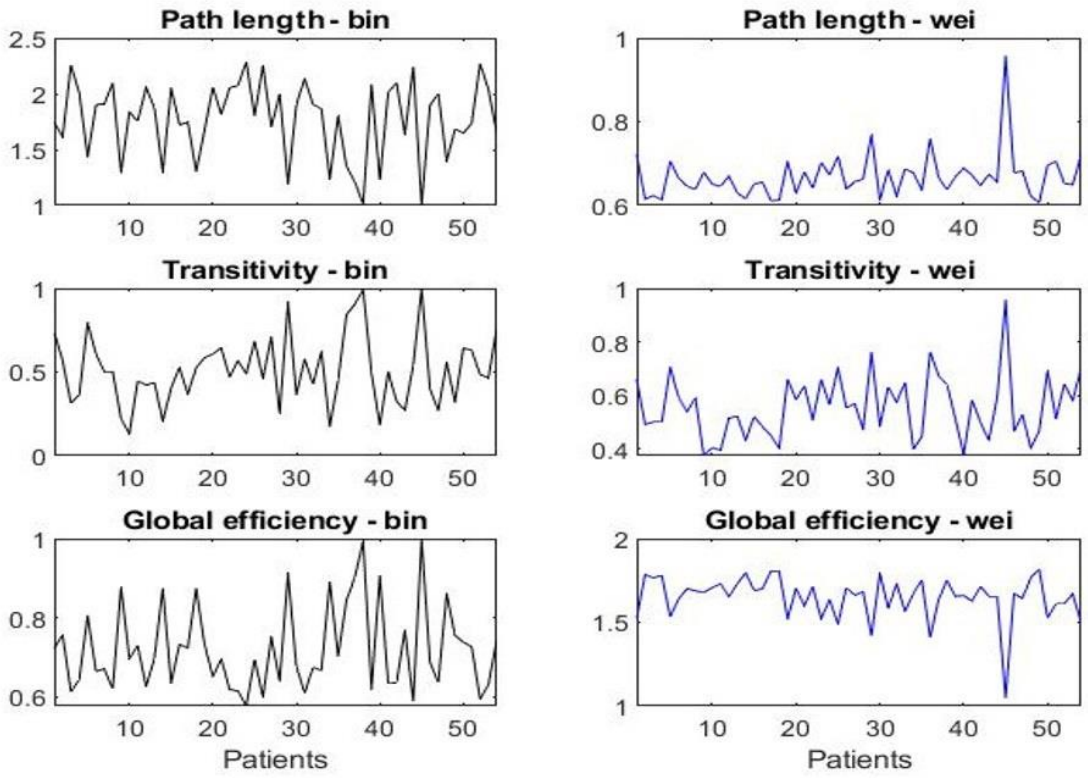


Figure 53. *Global indexes averaged across epochs, represented across subjects.*
This figure represents the indexes derived from a single correlation measure for demonstrative purposes, but the indexes were calculated for all the adjacency matrices corresponding to all the correlation metrics.
On the left, the indexes calculated from unweighted binarized matrices; on the right, the indexes calculated from weighted thresholded matrices.

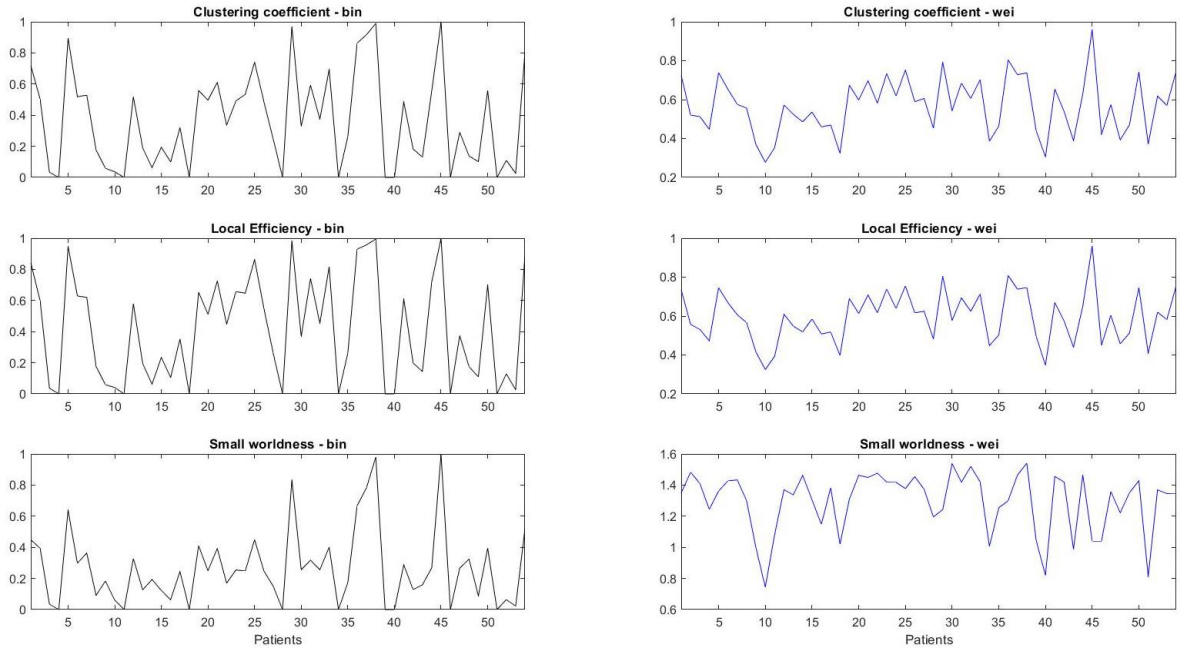


Figure 54. *Main Local indexes averaged across epochs, represented across subjects, from a single representative channel.*
This figure represents the indexes derived from a single correlation measure for demonstrative purposes, but the indexes were calculated for all the adjacency matrices corresponding to all the correlation metrics.
On the left, the indexes calculated from unweighted binarized matrices; on the right, the indexes calculated from weighted thresholded matrices.

Going into details, the path lengths (distance between nodes) and the **Characteristic path lengths** were calculated as functional integration indexes, allowing to quantify statistical connections between brain regions. **Global efficiency** was used as a complementary tool to the path lengths, as the former is not as influenced by infinitely distant nodes as the latter. **Clustering Coefficient**, on the other hand, was used as the main functional segregation index for individual nodes. **Transitivity** assumed the same role but generalized to the entire network. **Small-worldness** was used to quantify the network's balance between segregation and integration. The **Degree** was found to give a centrality measure to the network, in order to identify nodes communicating with many others. No hubs were individuated because the analysis was carried out using for either the whole network, or some specific channels, equal to all the subjects, and selected according to anatomical and functional regions of the brain involved in the primary topic, that is consciousness.

8.11 Comparisons across subjects

Once all the data have been processed and the measures obtained, the results were used to compare healthy and pathological subjects as follows:

For nodal measures, significative channels were selected as representatives. The values for these channels were compared across subjects.

Global measures were directly compared across subjects.

Boxplots were employed as a comparison means across **6 categories**:

- Healthy subjects
- Pathological subjects (all of them)
- Severity 1 pathological subjects
- Severity 2 pathological subjects
- Severity 3 pathological subjects
- Severity 4 pathological subjects

Nodal measures were also compared across subjects using **topological representations**.

In conclusion, all the obtained measures were subjected to significance tests: Lilliefors tests were employed to verify the normality of the distribution of the data, while a t-test (alpha= 0.05) was employed to reject the null hypothesis that data coming from normal distributions had mean difference equal to zero. The t-test was applied to compare healthy subjects to general pathological subjects. One-way and two-ways ANOVA tests were also employed, respectively across subjects and across both channels and subjects, comparing all subject categories.

8.11.1 Boxplots

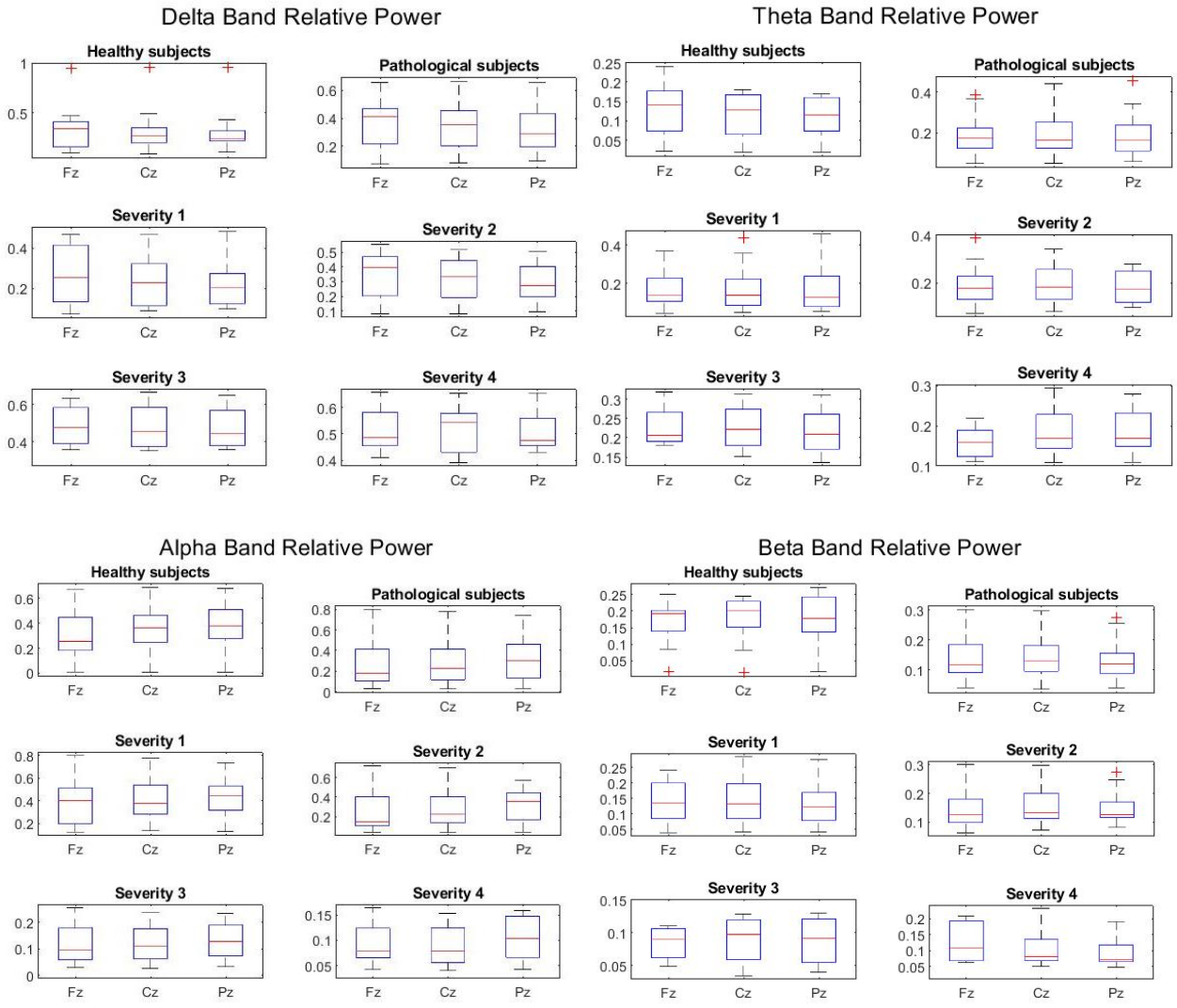


Figure 6. *Boxplots of Powers relative to the different frequency bands across representative channels, separating healthy subjects from all the pathological subjects and subjects belonging to to the 4 DOC severity levels.*

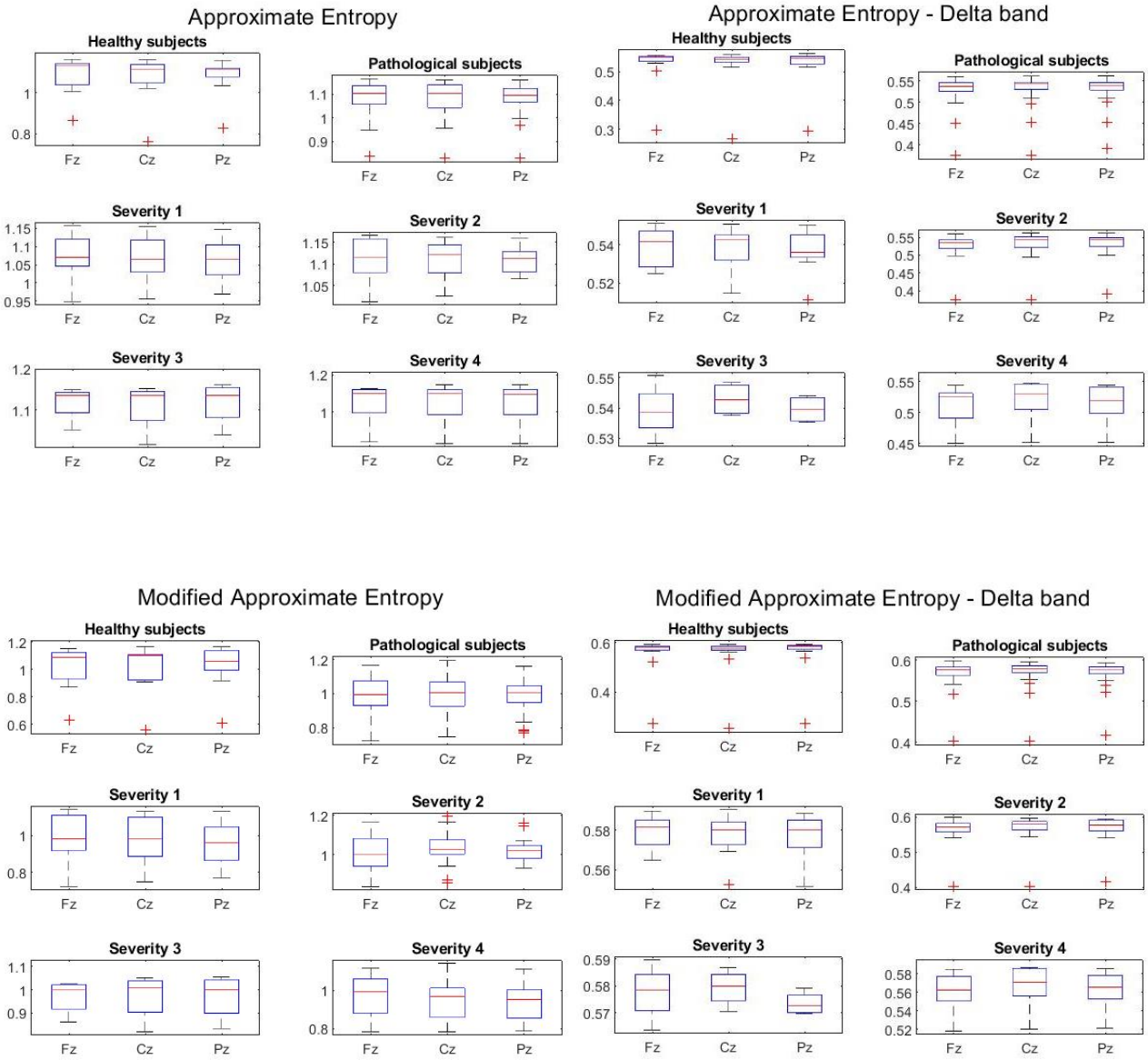


Figure 7. *Boxplots of the Original and Modified Approximate Entropy across representative channels, separating healthy subjects from all the pathological subjects and subjects belonging to to the 4 DOC severity levels.*

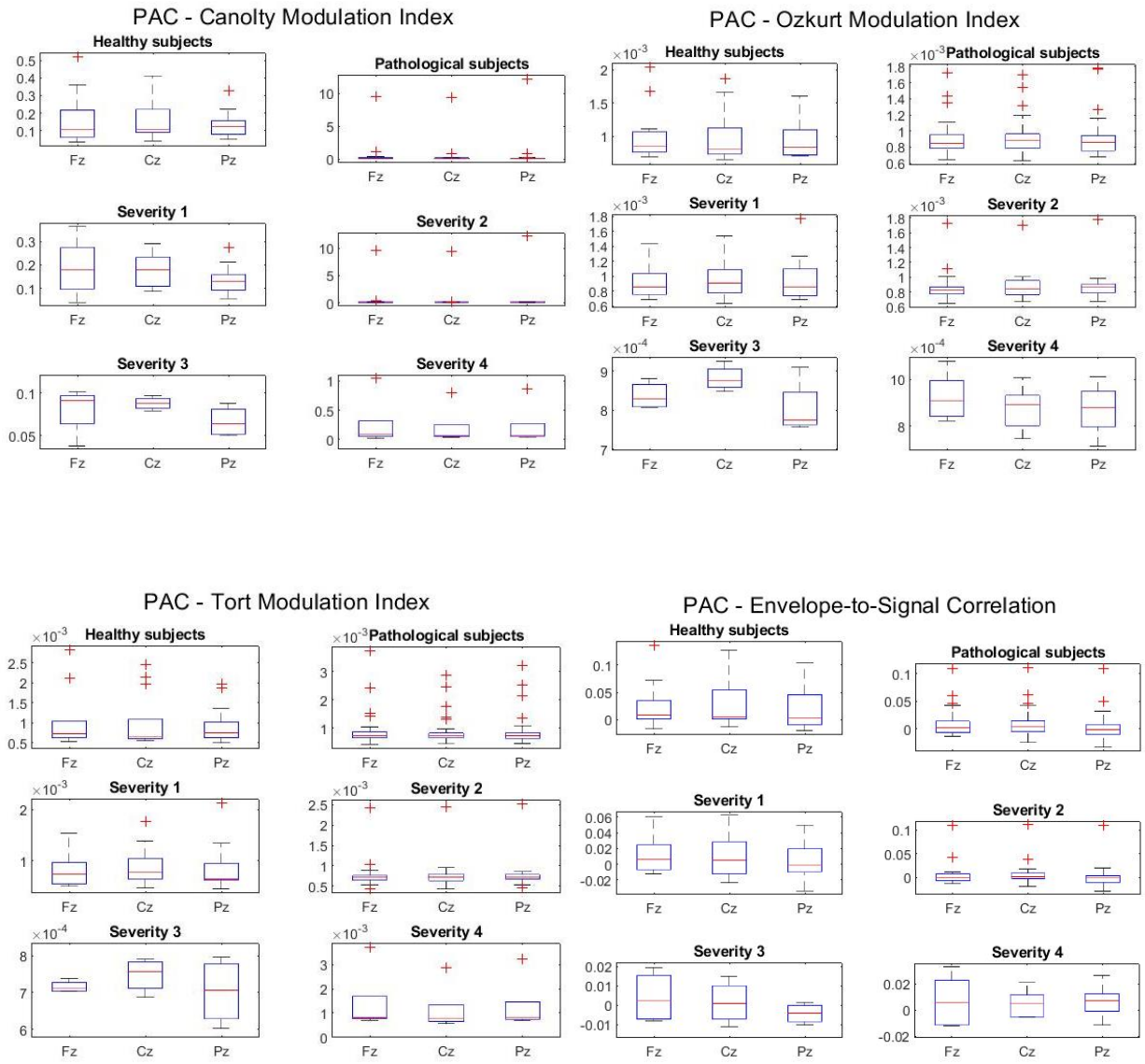


Figure 57*. Boxplots of the different PAC measures across representative channels, separating healthy subjects from all the pathological subjects and subjects belonging to to the 4 DOC severity levels.*

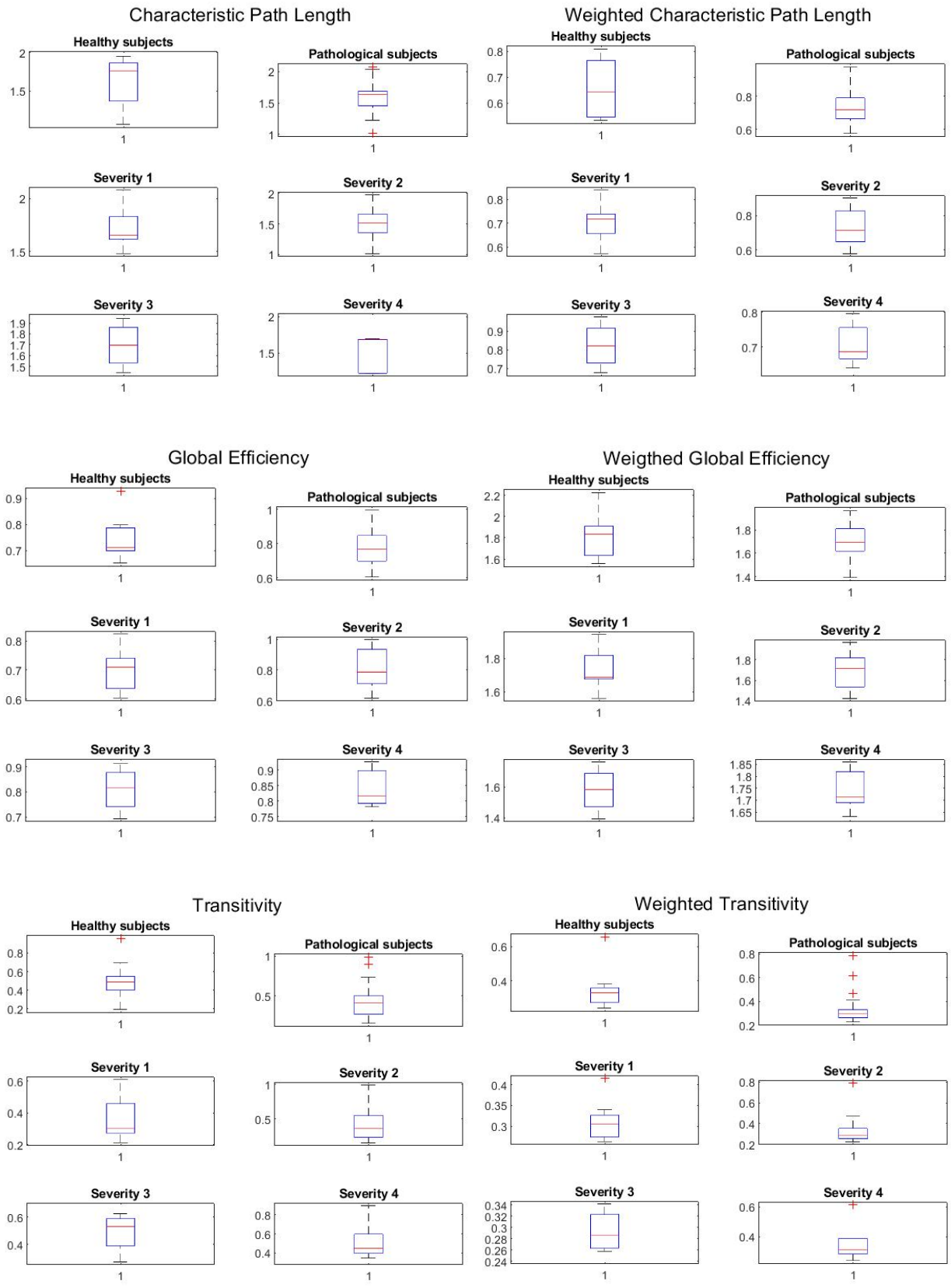


Figure 58. *Boxplots of the different Global FC indexes, obtained from both binarized and weighted adjacency marices, separating healthy subjects from all the pathological subjects and subjects belonging to the 4 DOC severity levels.*

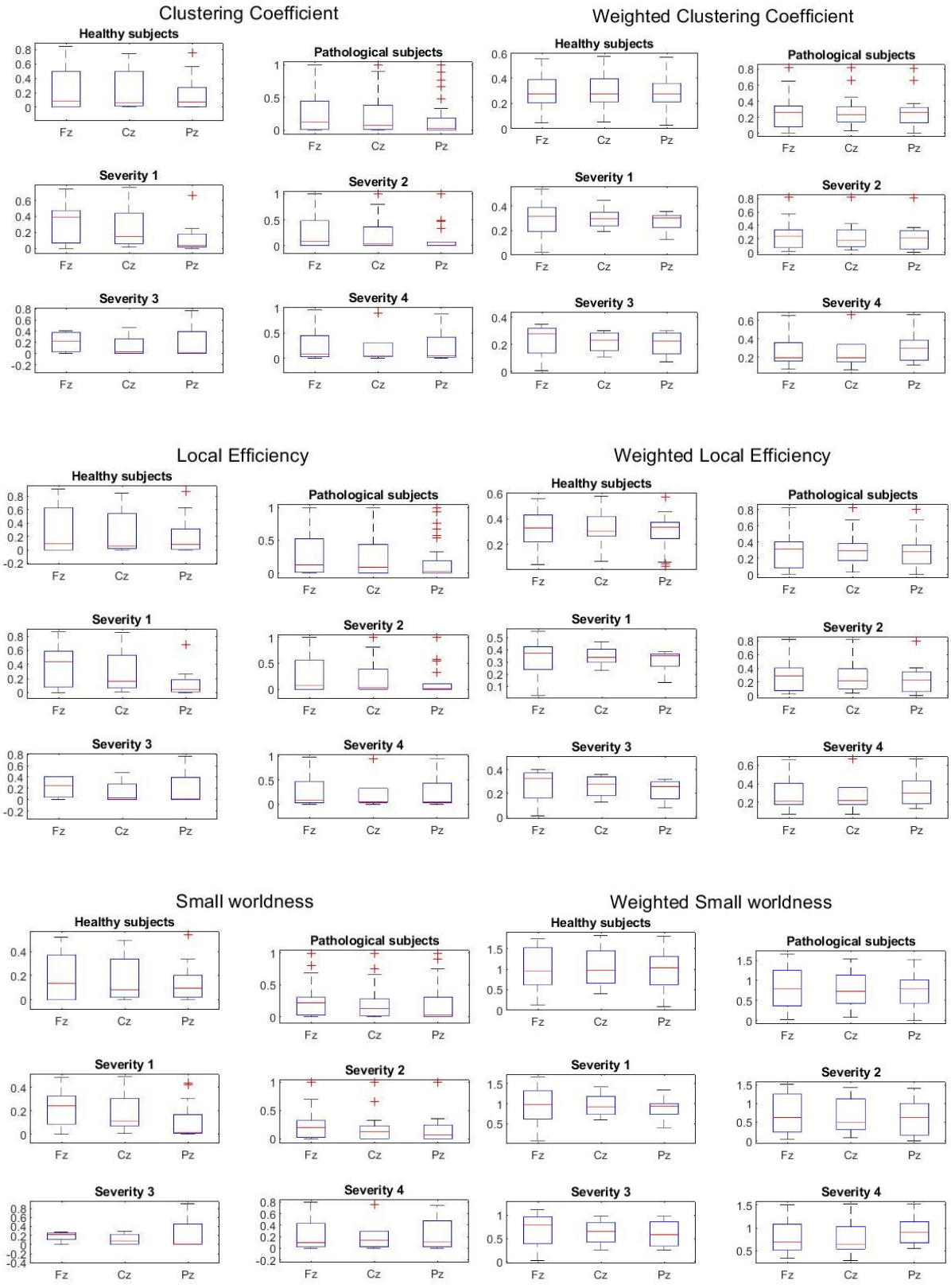


Figure 59. *Boxplots of the different Local FC indexes, obtained from both binarized and weighted adjacency marices, across representative channels. separating healthy subjects from all the pathological subjects and subjects belonging to the 4 DOC severity levels.*

8.11.2 Topographical representations

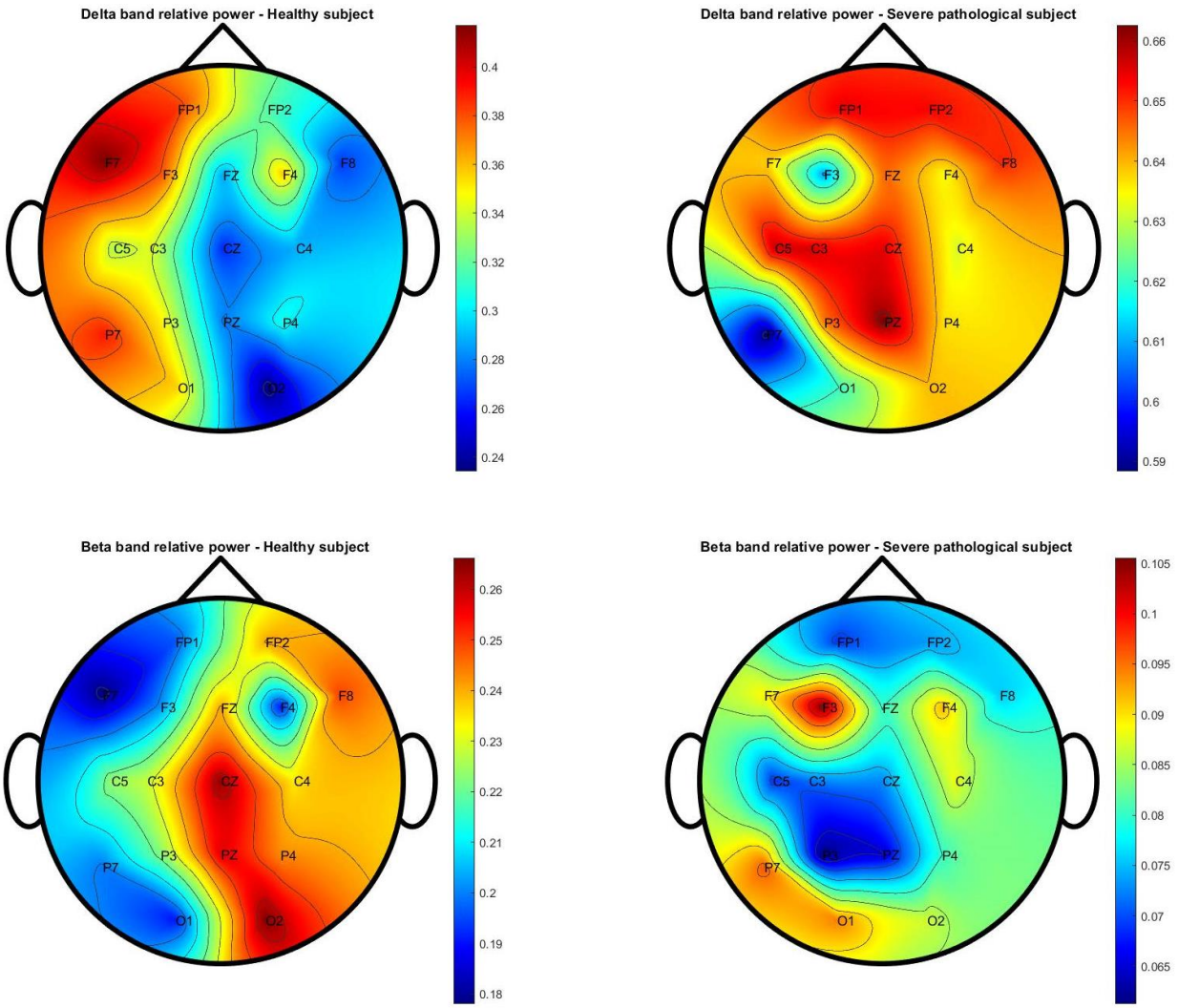


Figure 60. *Topography of Powers relative to the delta and beta frequency bands (the most informative), showing the differences between a healthy subject and a pathological subject belonging to the most serious DOC severity level.*

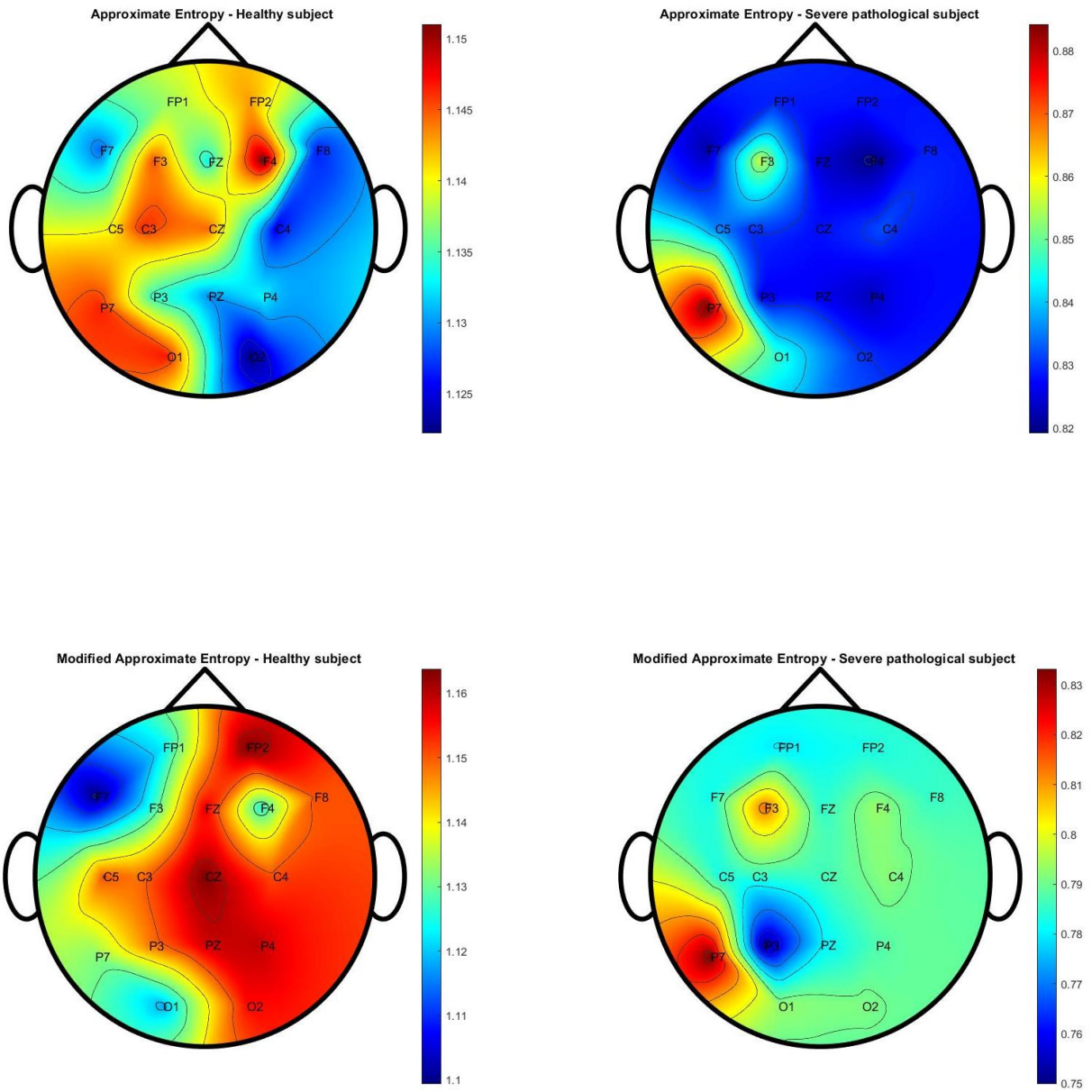


Figure 8. *Topography of the Original and Modified Approximate Entropy, showing the differences between a healthy subject and a pathological subject belonging to the most serious DOC severity level.*

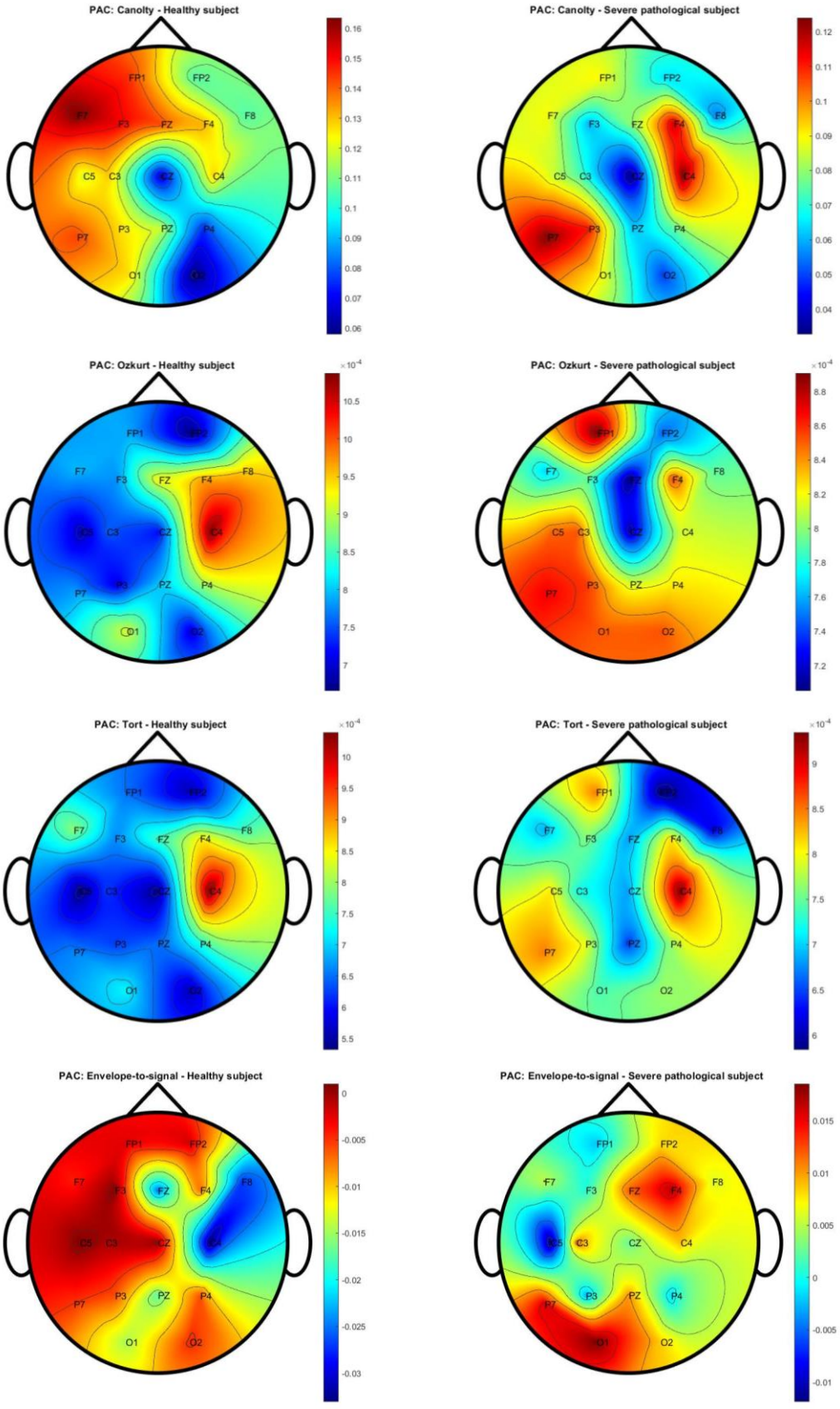


***Figure 62**. Topography of the different PAC measures, showing the differences between a healthy subject and a pathological subject belonging to the most serious DOC severity level.*

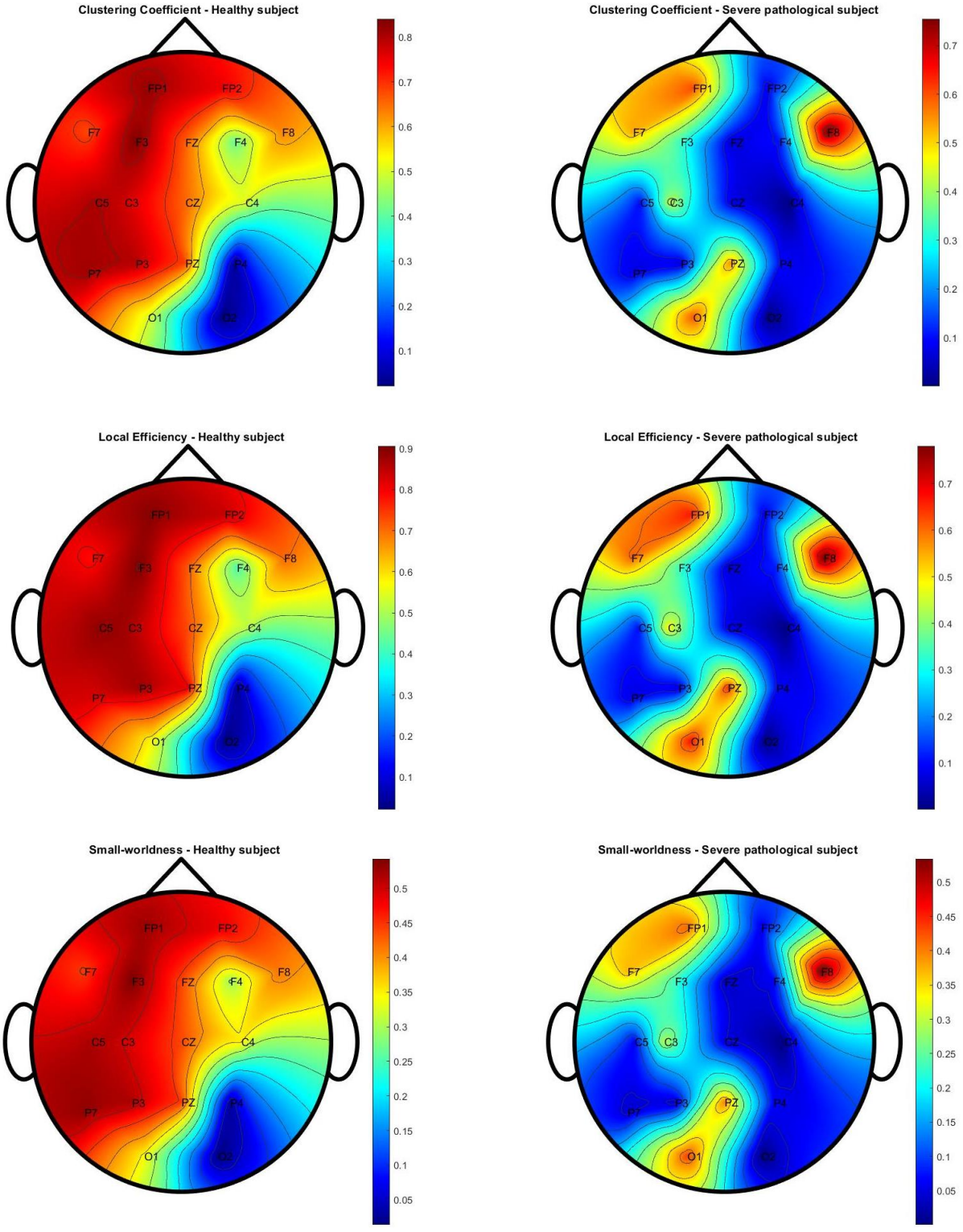


***Figure 63**. Topography of the different Local FC indexes, showing the differences between a healthy subject and a pathological subject belonging to the most serious DOC severity level.*

Conclusions

EEG signal, as a random process, is inevitably affected by its random nature, even more so when the subjects in question present pathological conditions such as those described in the proper sections. Several different approaches were employed for the analysis of this work, but due to the large amount of data collected on all the subjects, giving a univocal interpretation of the results was nearly impossible. Without a doubt, it was possible to individually analyze the results, comparing all statistical measures and functional connectivity indexes across subjects, especially separating the different subjects' categories.

The majority of the differences can be seen when comparing the healthy subjects' group to the whole pathological group, and also comparing the healthy subjects only to the most severe pathological ones. Intermediate states of DOC did not show the most significative results, mainly because of the uncertainties given by the brain injuries of those subjects. The most severe subjects gave more prominent results because in their case the brain status was more compromised.

In details, the first elements that were used for general comparisons were the powers relative to the different frequency bands: as shown in the figures in *Section 8.11*, Delta band resulted to be more present, in percentage, in pathological subjects, especially the ones in the most severe state. The condition of these subjects, in fact, causes a reduction of the waves at higher frequencies which instead appear in awake subjects. As a confirm, Alpha and Beta bands resulted nearly absent for pathological subjects.

Regarding to the main object of interest of this work that is functional connectivity, the most informative indexes were the Characteristic Path Length and the Global Efficiency for the Global measures, and the Clustering Coefficient and the Local Efficiency for the Local measures. This was reassuring because similar studies conducted on different conditions reported these indexes as the most valuable. In general, healthy subjects showed higher connection levels with more stable values, while the pathological ones showed either lower values, higher standard deviations and a large number of outliers. The use of both weighted and unweighted networks allowed to overcome the limits of the two: the unweighted networks helped simplifying the problem, but the weighted ones allowed to preserve all the information. Furthermore, the nodes of the networks were made to match the electrodes, so every region of the brain could be mapped by its corresponding channel.

The same considerations can be made regarding to statistical means such as Approximate Entropy and PAC. The Modified formulation of ApEn, in addition, resulted to be more stable in the results, but both the Original and the Modified ApEn were highly affected by the analyzed frequency band. Delta band was the most informative because common to all subjects, both healthy and pathological. Beta band, though, was used as control because barely present in pathological subjects. In this regard, PAC measures resulted to be valuable means, precisely because of the selected frequency bands. Also in this case, comparing Delta to Alpha band served to visualize the differences between subjects, while the Alpha and Beta band comparison served as control.

The calculated measures resulted to have intermediate significance levels: the significance tests were not always passed because, given the reduced number of subjects especially for certain categories, the distribution of the values was not always normal. Furthermore, data belonging to pathological subjects were affected by the condition itself, as every injury could lead to different results in different brain portions. This is the reason for which, in general, comparisons between single pairs of subjects were preferred. The topographical figures comparing healthy and severe pathological subjects served as representations of the trend of the measures across all channels, showing major differences independently from the brain region.

Future applications could employ variations and additions to the methods used in this work: the whole MATLAB code might be optimized, especially considering that the heaviest computations, for every combination of frequency band, channel and subject, required several hours each. Going into details, the brain's network might be mapped from regions instead of channels through the reconstruction of the signal source. This way, the mapping would correspond to specific functional regions of the brain rather than to simple anatomical positions. Approximate Entropy was studied to comprehend the predictability over time of signals individually taken, but any form of Cross-Entropy could be employed to see how signals influence each other, and thus, how a brain's region is affected by others. Different correlation measures were used, so the most adequate to the case could be selected for an in-depth analysis. Consequently, the appropriate threshold values for connectivity matrices could be found and used to get more reliable value of the connectivity indexes.

This work's main target was to gather data to manually characterize subjects according to the categories given by their conditions. In future prospects, A.I. algorithms like Machine Learning's Support Vector Machines and Deep Learning's Neural Networks could be developed to automatically classify subjects. This way, the classification of the subjects would become possible even with a large number of data and the consequent statistical analysis would certainly be more reliable.

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